Bioavailability, bioefficacy and wash resistance

Bioavailability, bioefficacy and wash resistance

What about the surface chemistry should we be measuring along the life cycle of ITNs, and what methods do we need to measure it?

Dr Rosemary Lees and Assoc Prof Corine Ngufor

Setting the Scene

Setting the scene

Relevant issues arising from the PQT/VCP Product Review Report

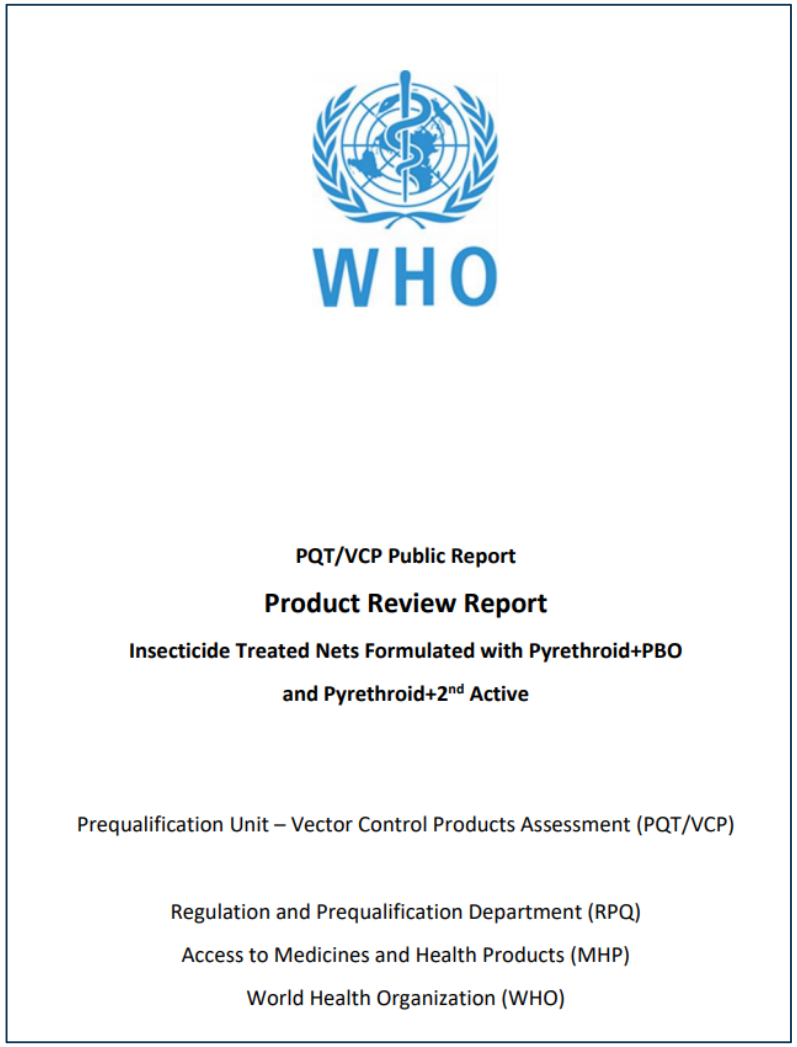
Introducing presenters for the session

Key questions to address in this session

Relevance of Surface Chemistry to ITNs

- Quantification of AI or synergist on the surface of the fibres
  - Dynamic – AI released from the reservoir to the surface, regenerates after washing
- Presentation of AI or synergist
  - Physical state (amorphous or crystalline)
  - Crystalline particle size and shape
  - Distribution of AI or synergist on the surface of the polymer
  - Bio-available fraction of AI / synergist ⟶ Pick up by mosquito

PQT/VCP Product Review Report of pyr+ ITNs



WHO

PQT/VCP Public Report

Product Review Report

Insecticide Treated Nets Formulated with Pyrethroid+PBO and Pyrethroid+2nd Active

Prequalification Unit – Vector Control Products Assessment (PQT/VCP)

Regulation and Prequalification Department (RPQ)

Access to Medicines and Health Products (MHP)

World Health Organization (WHO)

PQT/VCP Product Review Report of pyr+ ITNs

- Methodological issues highlighted:
  - “data… often generated in a manner which deviated from the intent of the product, possibly in order to align with the standards/methods… Product testing should be conducted to support the use of the product.”

  We should be measuring criteria that relate to performance and criteria that reflect the specific nature of the net

  - “Data requirements and methodology for the Wash Resistance Index should be reviewed to consider the impact of selected wash intervals.”

  Regeneration times should be reviewed and linked to product performance

  - “there needs to be a defined distinction between bioassays for the purpose of characterization of quality related information… and testing to investigate efficacy.”

  Different methods may be needed and different endpoints measured for QC and for efficacy testing

PQT/VCP Product Review Report of pyr+ ITNs

- Formulation issues relevant to surface chemistry:
  - "… a product which delivers continuous and controlled release of the active substance(s)…"

  Formulation is critical to bioefficacy as it relates to presentation and bioactivity of the AI
  - "…changes to source materials, formulations and manufacturing processes may limit the usefulness of historic data."

  Therefore, any changes need to be considered for their impact on performance
  - "approved storage conditions and maximum storage period" and "validation of accelerated storage methods"

  Surface chemistry should be considered in validating storage conditions and times

Current Guidelines on Washing

- Wash Resistance Index and Regeneration Times

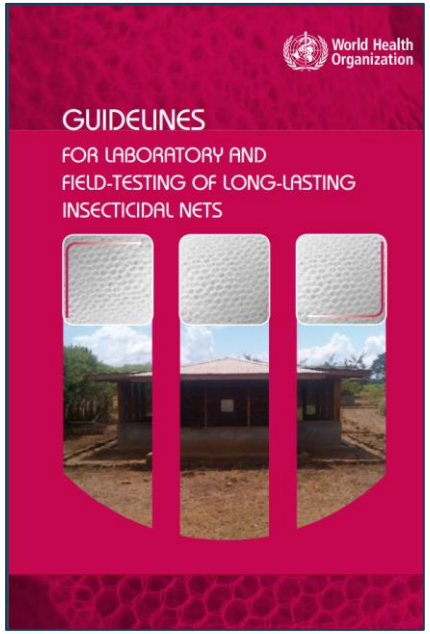


Table 1. Main parameters assessed in phase I, II and III studies of long-lasting insecticidal mosquito nets

Phase	Type of study	Parameters measured
I	Laboratory	Regeneration of insecticidal activity
		Efficacy and wash-resistance
II	Small-scale field trial	Wash-resistance
		Efficacy as measured by vector mortality and blood-feeding inhibition
III	Large-scale field trial	Long-lasting insecticidal efficacy
		Rate of loss or attrition of nets
		Physical durability of netting material
		Community acceptance
		Safety

$$w = 100 \times \sqrt[4]{(t_4/t_0)}$$

where: w = wash resistance index, expressed as a percentage; t_4 = total active ingredient content (in g/kg) after 4 washing cycles; and t_0 = total active ingredient content (in g/kg) before washing (no washing).

2.3 Efficacy criteria for phase I studies

Nets washed at least 20 times that meet the criteria of WHO cone bioassays (≥ 80% mortality or ≥ 95% knock-down) or of the tunnel test (≥ 80% mortality or ≥ 90% blood-feeding inhibition) meet the criteria for undergoing phase II testing.

3.11 Efficacy criteria for phase II studies

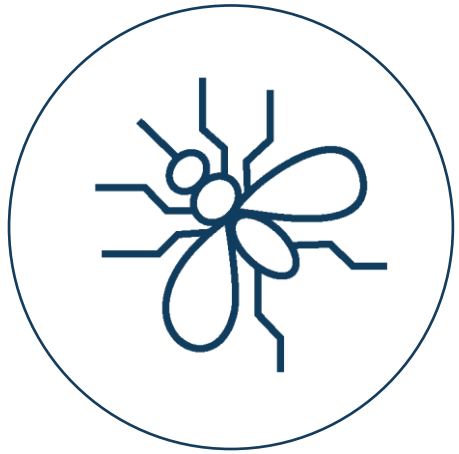
A candidate LN is considered to meet the phase II efficacy criteria if, after 20 washes, it performs as well as or better than the reference LN when washed 20 times in terms of blood-feeding inhibition and mortality. Such candidate LNs are given an interim recommendation.

Introducing our Presenters

- **Svetlana Ryazanskaya**, IVCC - Relevance of Chemical Assessments for ITN
- **Bart Kahr**, NYU – polymorphism, blooming and inhibiting crystallization, with bioefficacy data from LSTM
- **Seth Irish**, Swiss TPH – Cone bioassays for estimating bioefficacy in quality assurance
- **Ole Skovmand** – Two proposed methods to measure bioavailable surface AI
- **Moussa Cisse**, LBMA-USTTB - C-vue HPLC method

Key Questions for this session

- **What surface chemistry characteristics would be informative?**
  - Critical to efficacy and durability, and product performance
  - What to measure when? Product development, demonstrating bioefficacy, predicting efficacy, defining specifications, quality assessment including durability monitoring…
- **What methods should be/are being developed?**
  - What questions do we need them to address?
  - Where should our focus be?
  - Identifying, developing and validating informative methods
- **What research questions should be addressed to support this work?**
  - Ongoing or gaps to be filled



Thank you

PQT/VCP Product Review Report of pyr+ ITNs

- Why are we discussing Surface Chemistry?
  - “The physical/chemical data requirements… should be reviewed with particular attention paid to meaningfulness/value of each current data requirement, gaps in information, availability of methodology, potential need for establishing attribute specific standards.”
  - “The focus of chemistry assessments should be shifted from total AI/synergist content to surface concentration and further that which is bioavailable.”
  - “Method development for direct measurement of surface concentration should be a longer-term goal with the use of modelling of expected surface concentrations until direct methods are available.”
  - ITN specifications “could include physical and chemical properties that are critical to efficacy and durability…”
  - “Consideration should be given to development of testing to assess surface concentrations, which parameters are critical to physical durability, and whether a single measure of wash resistance is appropriate for ITNs.” – also presentation of chemistry?

PQT/VCP Product Review Report of pyr+ ITNs

- Recommendations for improved guidance on:
  - Study designs for wash regeneration, wash resistance, and Experimental Hut Trials (EHTs), including statistical power calculations
  - Selection of mosquito strains to be used in bioassay and efficacy testing - resistance mechanisms, intensity, or other characteristics relevant to the intended action of the ITN
  - Selection of meaningful methods and endpoints which are appropriate and relevant to the investigation of the intended action of the ITN. This should include guidance on the weighting of different endpoints to improve the interpretation of the generated data.
  - Purpose of positive and negative controls in bioassays, as well as the interpretation of control data for informing the validity of the study and correction of test results (e.g., control corrected mortality)
  - Reporting of bioassay and efficacy data that captures the variability of test methods and products and standardises result reporting accordingly.
  - Where to use cone tests, tunnel tests, or both

Relevance of Chemical Assessments for ITN

Relevance of Chemical Assessments for ITN

Dr Svetlana Ryazanskaya Randle
May 2022

Introduction – ITN product lifecycle

Storage

Distribution

Use in people's home

R&D Formulation Development

Manufacturing

KEY INDICATORS:

chemical interactions

insecticidal dosage/stability, migration/regeneration/chemical durability

insecticidal presentation

NET PERFORMANCE

IVCC

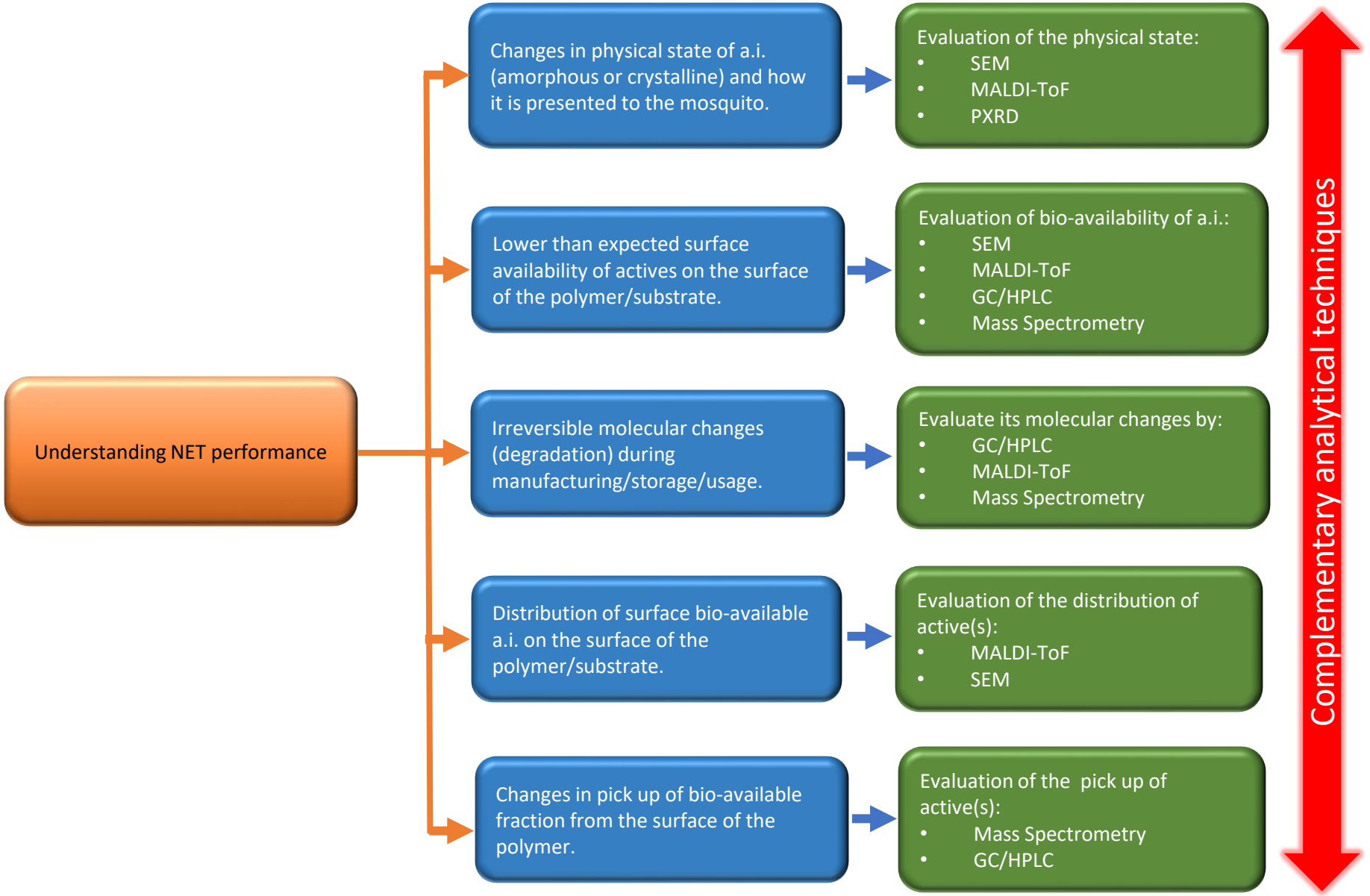
ITN chemical assessment – total *vs* surface

Net performance

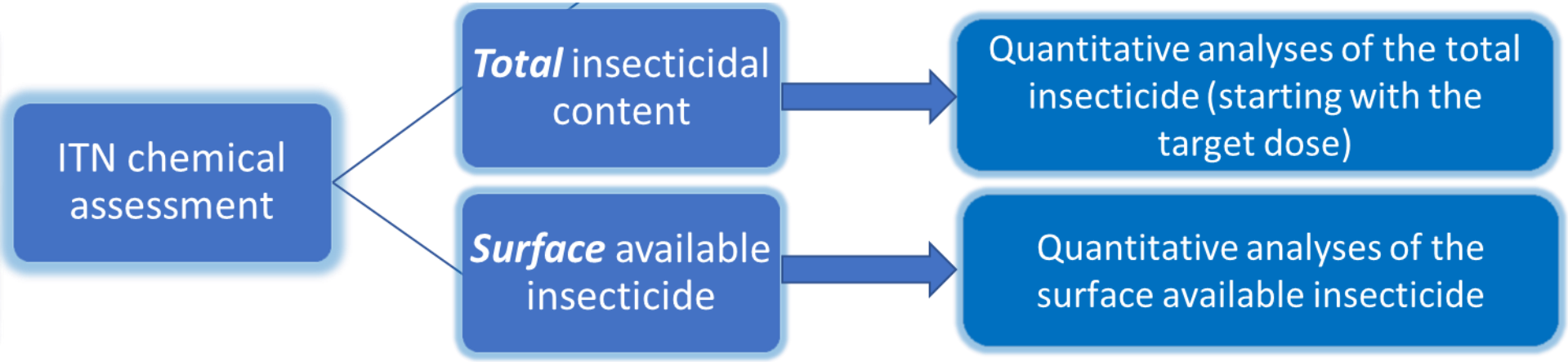
ITN chemical assessment

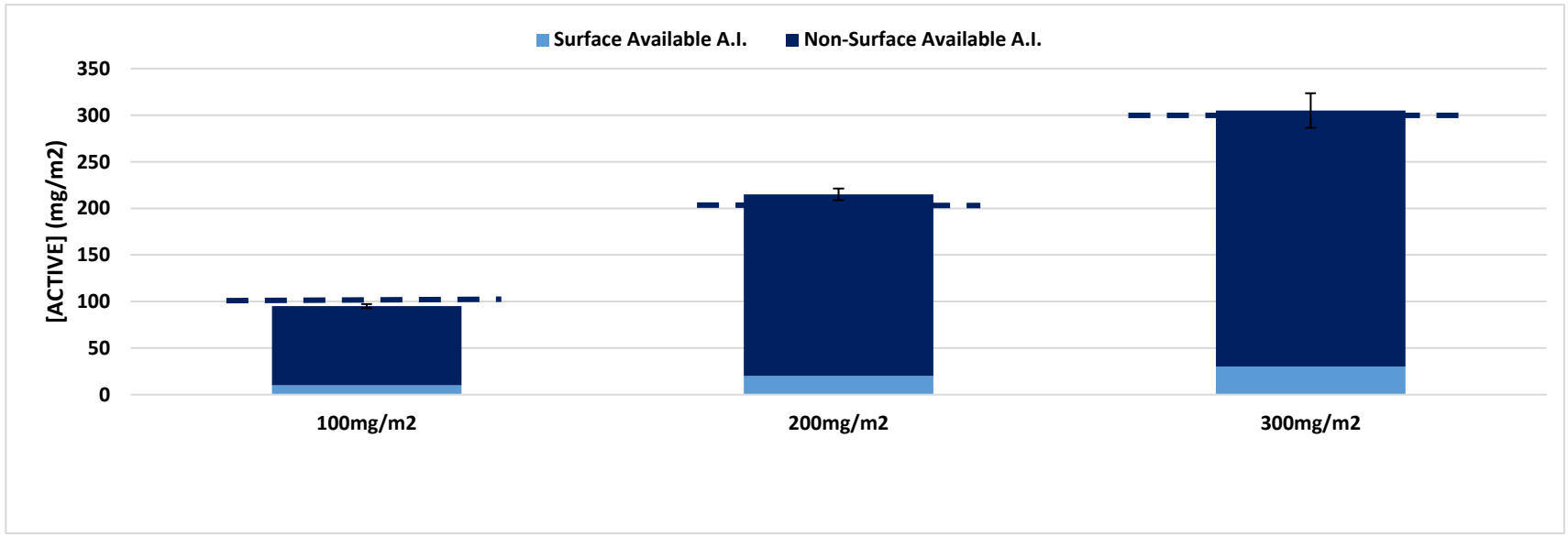
- ***Total*** insecticidal content → Quantitative analyses of the total insecticide (starting with the target dose)
- ***Surface*** available insecticide → Quantitative analyses of the surface available insecticide
- Distribution of ***surface*** available insecticide → Qualitative analyses of insecticides on the surface of the polymer
- Presentation of ***surface*** available insecticide → Qualitative analyses of physical presentation of insecticides on the surface of the polymer
- ***Pick up*** of bio-available insecticide → Quantitative analyses of mosquito extracts

Product related questions



ITN chemical assessment – total *vs* surface active content example data





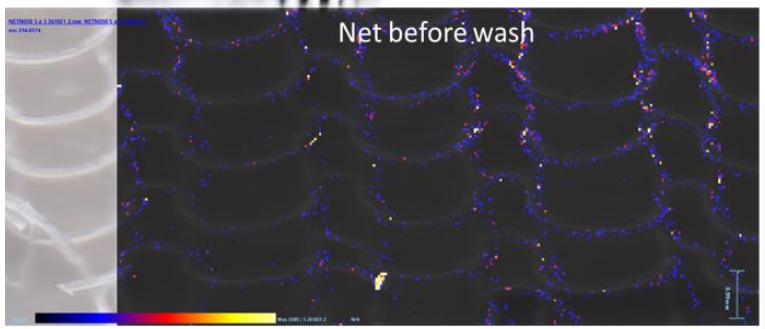
HPLC analysis of total insecticide content vs surface available insecticide at different target dosages (dotted line).

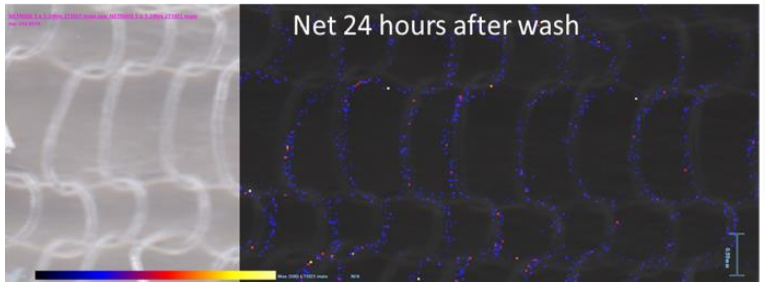
Quantitative analyses:

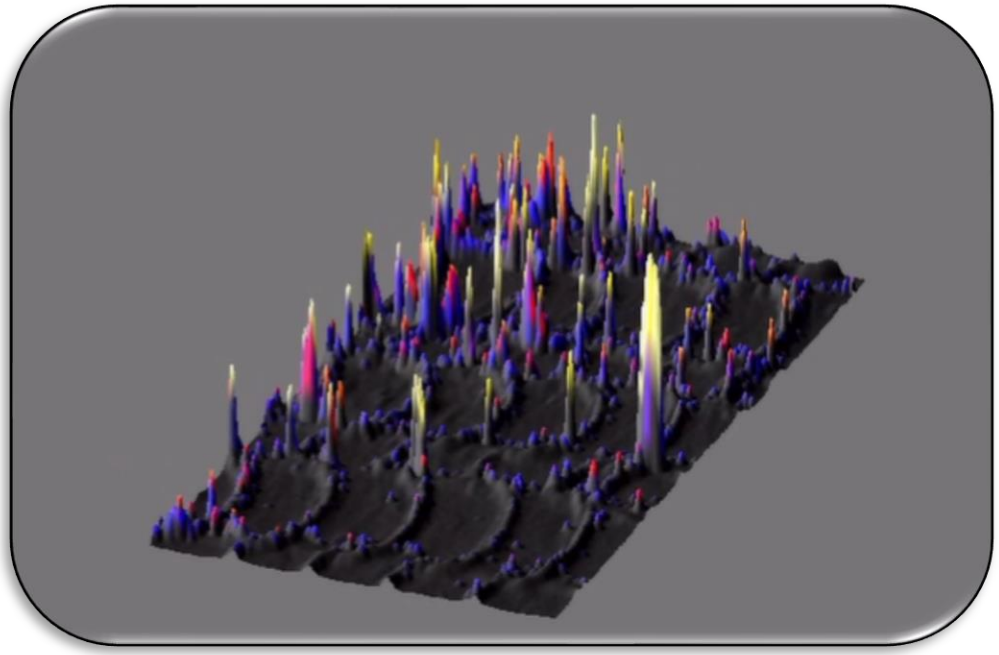
- Total active content (target dosage)
- Surface available content
- Over time (washability, regeneration, storage, use)
- Different formulations
- Correlation with bio-efficacy and surface presentation, distribution and availability

ITN chemical assessment – active distribution on the surface of the polymer example data

ITN chemical assessment → Distribution of ***surface*** available insecticide → Qualitative analyses of insecticides on the surface of the polymer







Qualitative analyses:

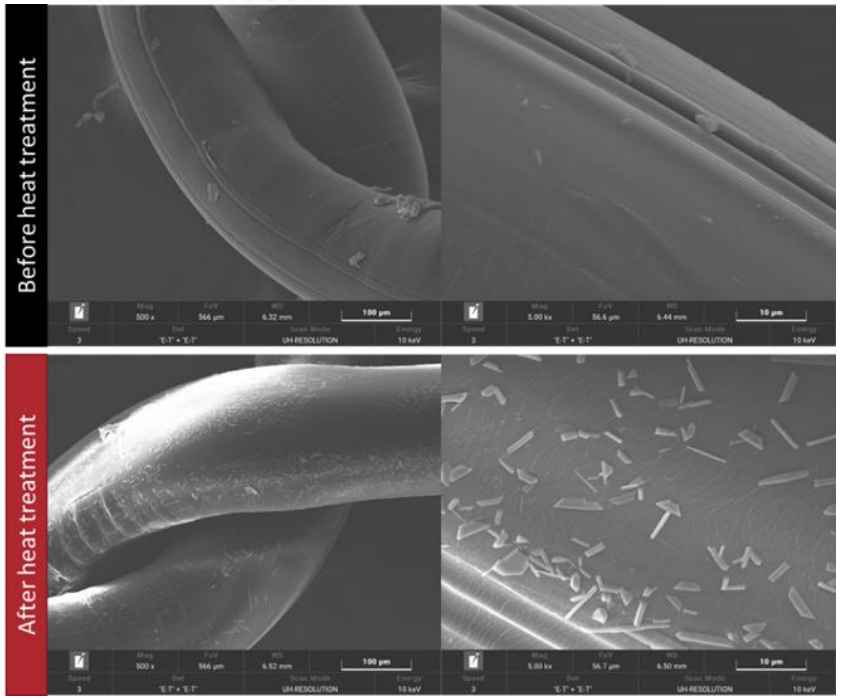
- Distribution of active(s) on the surface
- Over time (washability, regeneration, storage, use)
- Using different formulations
- Correlation with bio-efficacy and surface presentation, distribution and availability

Imaging MALDI-ToF analysis of active on the surface of the polymer before the wash and 24 hours after wash. Optical images of the nets aligned with 2D images (distribution of active) and 3D images (mass spectral fingerprints, m/z intensity) of the active detected on the surface of the polymer.

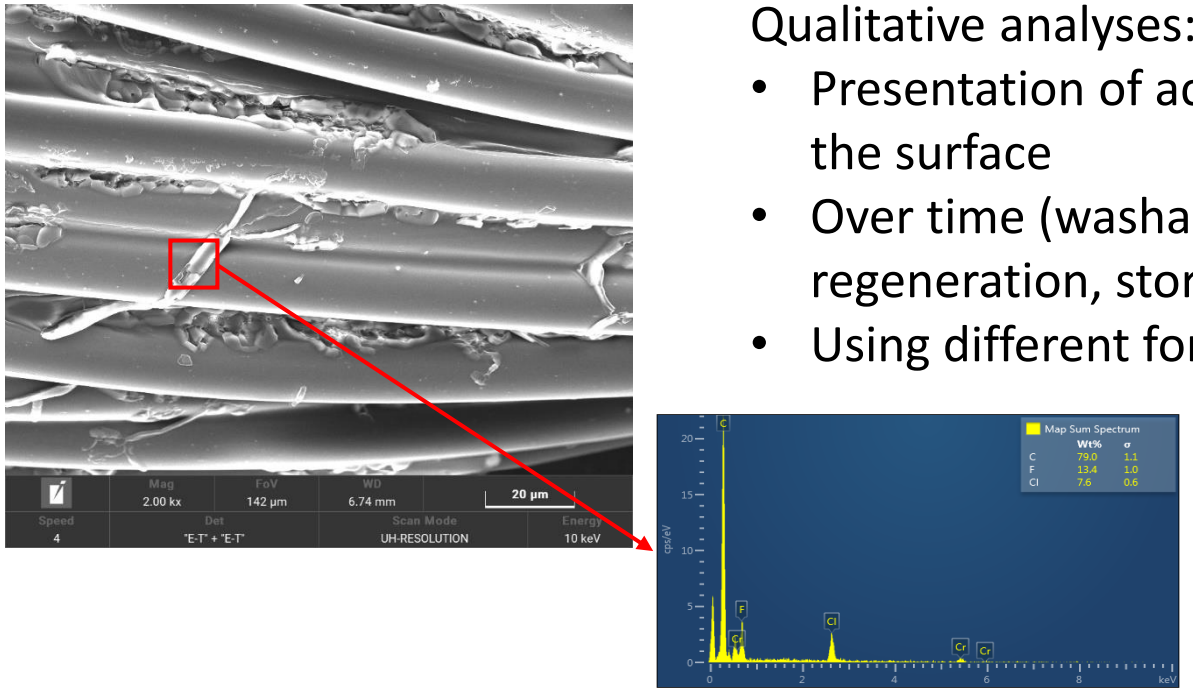
ITN chemical assessment – active presentation on and in the polymer example data

ITN chemical assessment → Presentation of ***surface*** available insecticide → Qualitative analyses of physical presentation of insecticides on the surface of the polymer

A



B



Qualitative analyses:

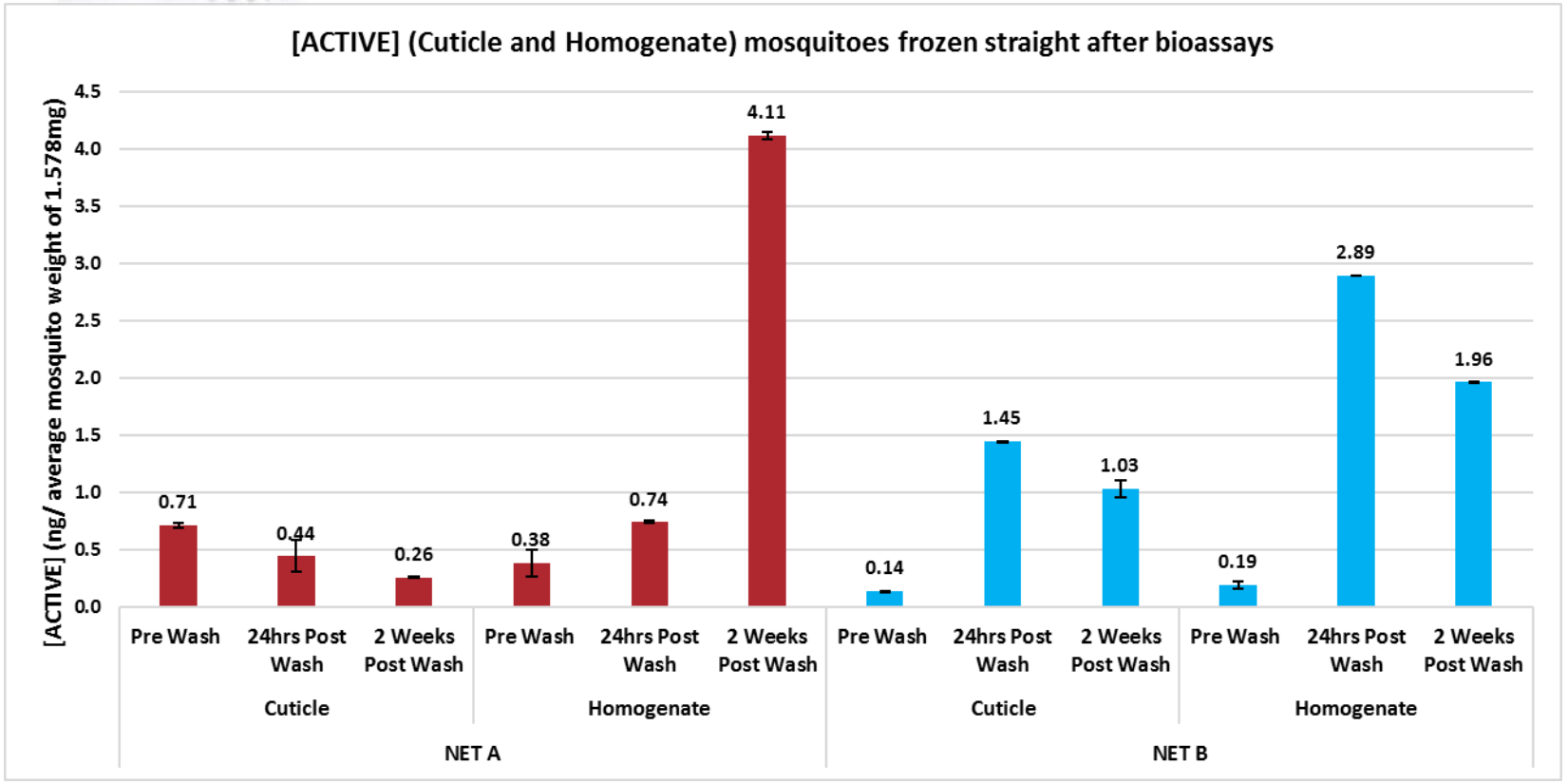
- Presentation of active(s) on the surface
- Over time (washability, regeneration, storage, use)
- Using different formulations

[A] - SEM analysis of incorporated ITN, where the presentation of crystalline active is assessed before and after heat stress at 54 °C for 7 days.

[B] - SEM analysis of coated ITN with EDS fingerprint of active on the surface of the polymer.

ITN chemical assessment – active pick up by mosquito example data

ITN chemical assessment → ***Pick up*** of bio-available insecticide → Quantitative analyses of mosquito extracts



Quantitative analyses:

- Mosquito cuticle
- Mosquito homogenates
- Over time (washability, regeneration , storage, use)
- Exposure time - 3, 30, 60 minutes, overnight
- Different formulations (Net A *vs* Net B)
- Correlation with bio-efficacy and surface presentation, distribution and availability

Food for thought

Laboratory tools

Field tools

Affordable
Non-destructive
While in use
Hand-held
Real-time measurement

Thank you

Funding Partners

Bill & Melinda Gates foundation

UKaid from the British people

Australian Aid

The Global Fund To Fight AIDS, Tuberculosis and Malaria

USAID FROM THE AMERICAN PEOPLE

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Agency for Development and Cooperation SDC

Unitaid Innovation in Global Health

www.ivcc.com

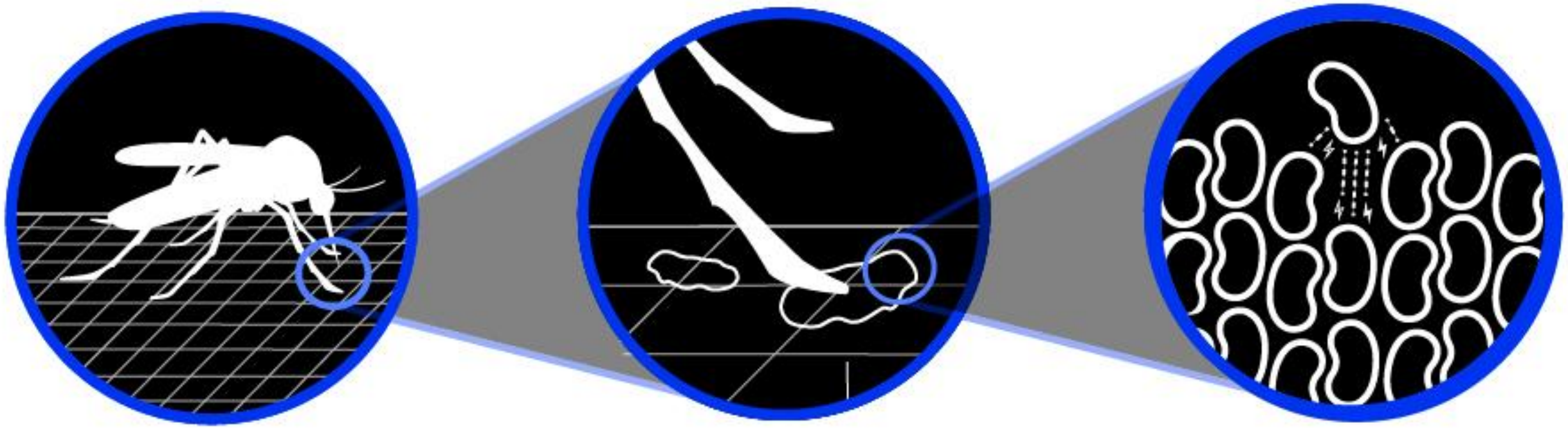
ivcc-innovative-vector-control

invectorcontrol

invectorcontrol

invectorcontrol

Mosquito Meets Crystal



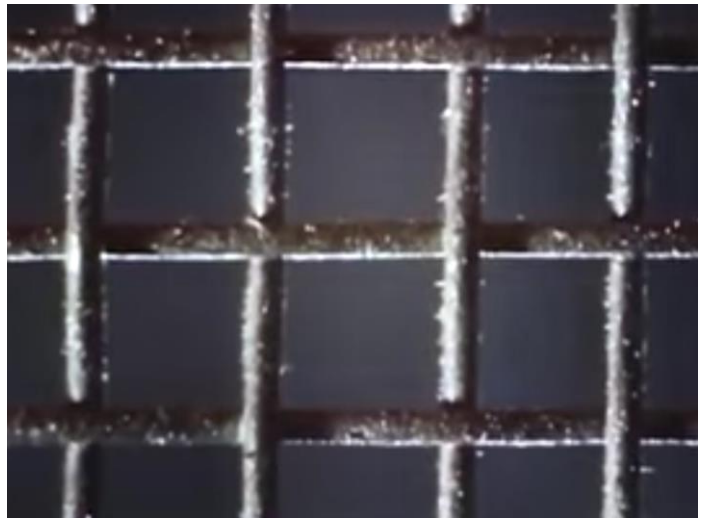
Mosquito Meets Crystal

Bart Kahr, Michael Ward, Bryan Erriah, Leilani Smith, Alexander Shtukenberg
New York University

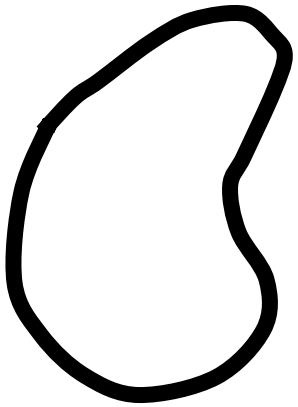
Liverpool School of Tropical Medicine
19 May 2022

1946 Sherwin-Williams promotional film

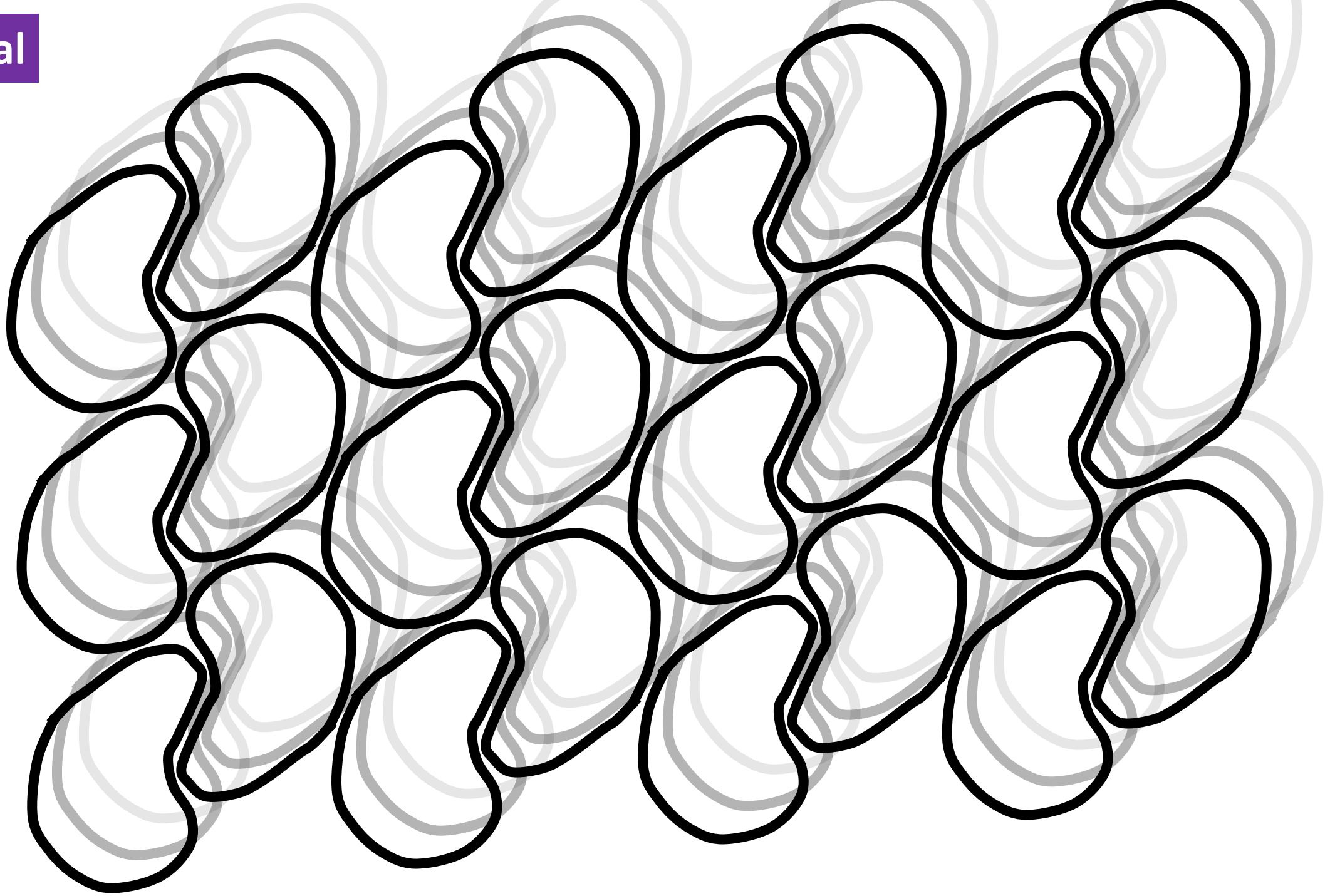
YouTube



Molecule

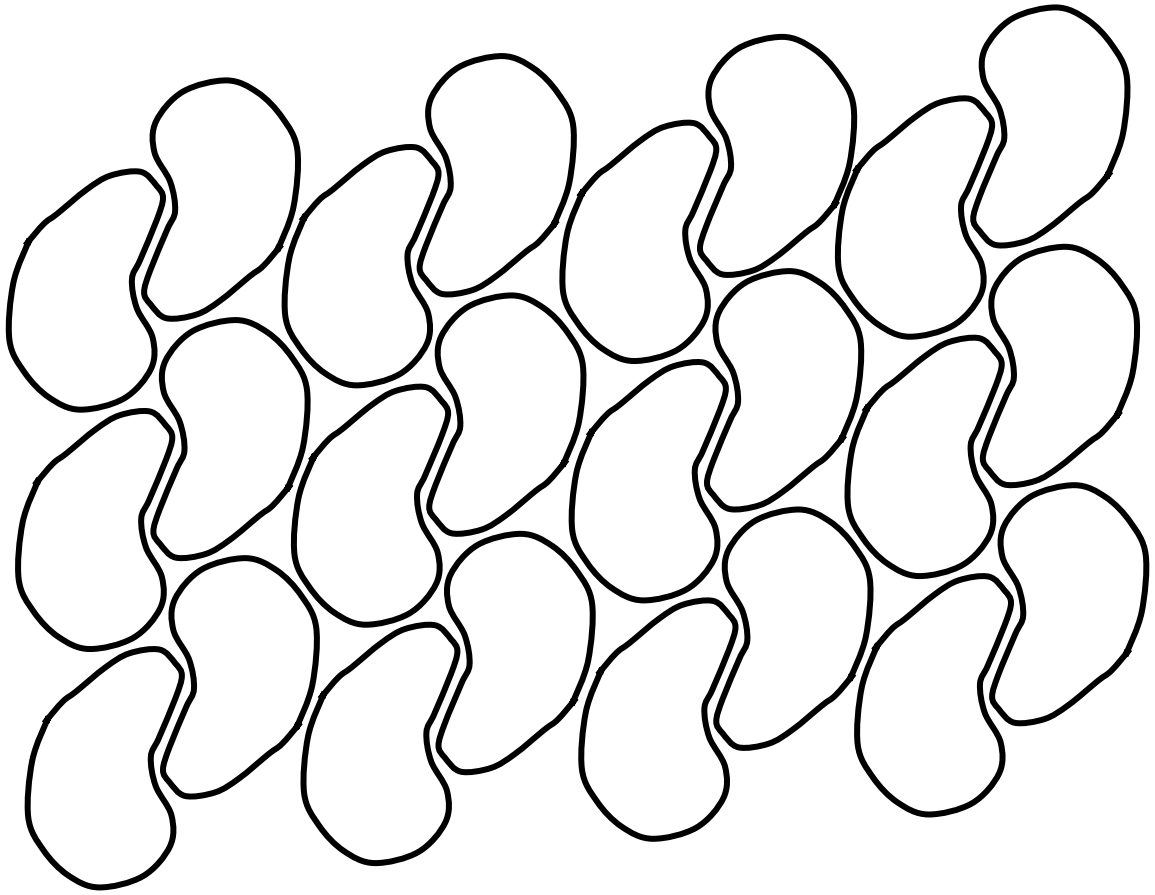


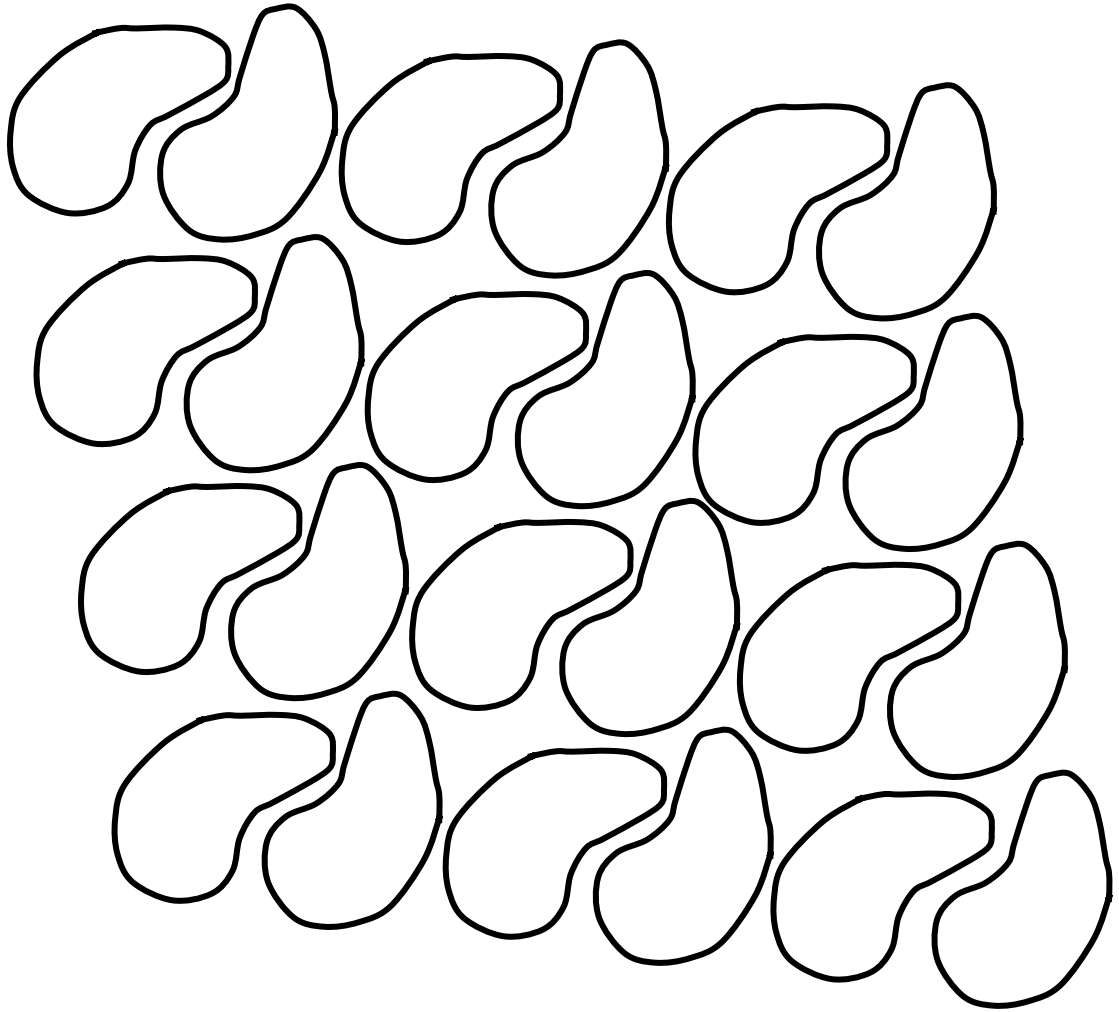
Crystal



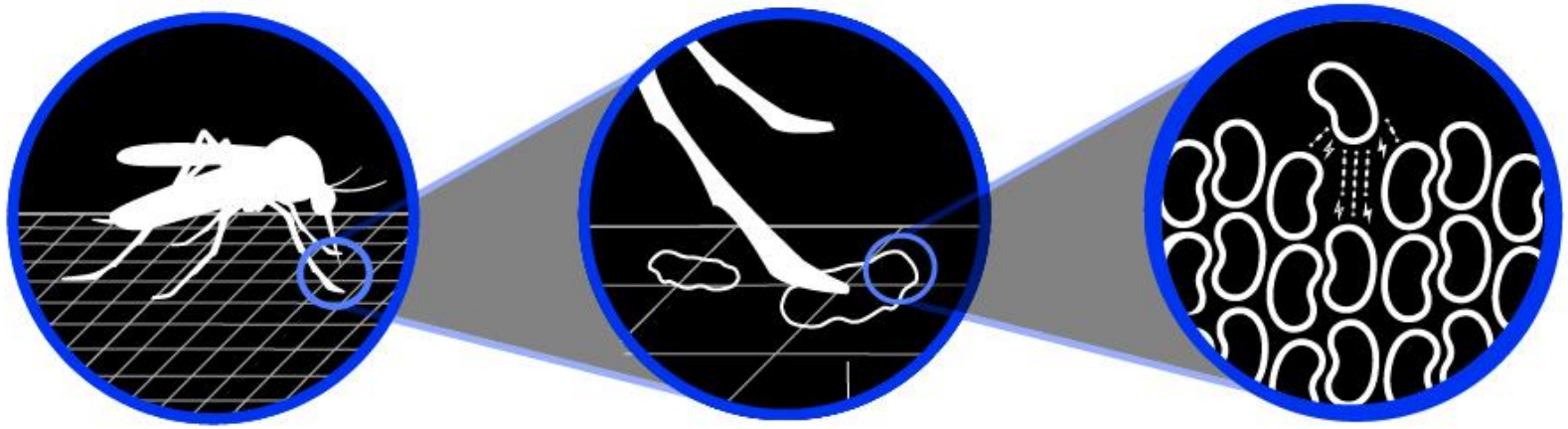
Polymorphism: Different arrangements of molecules in crystals

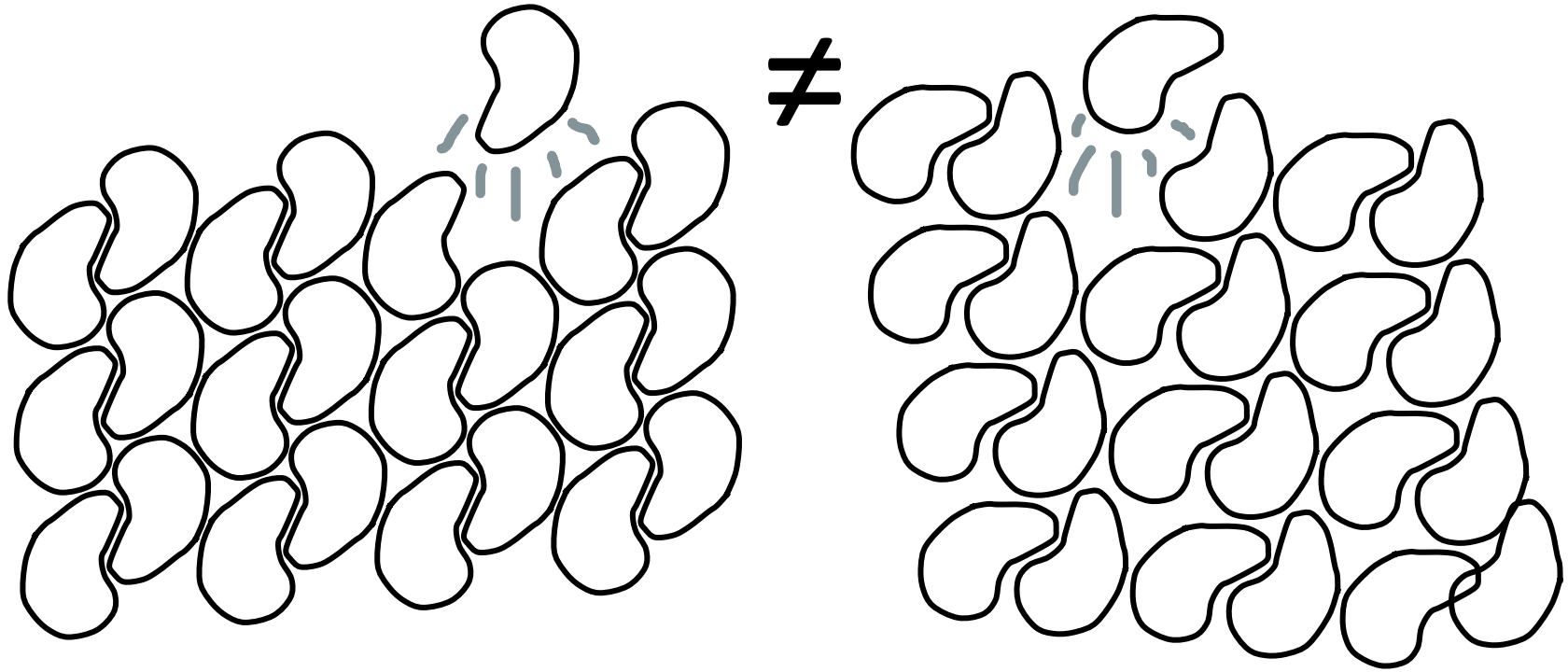
- Ubiquitous
- Hard to predict
- Hard to control
- Consequential



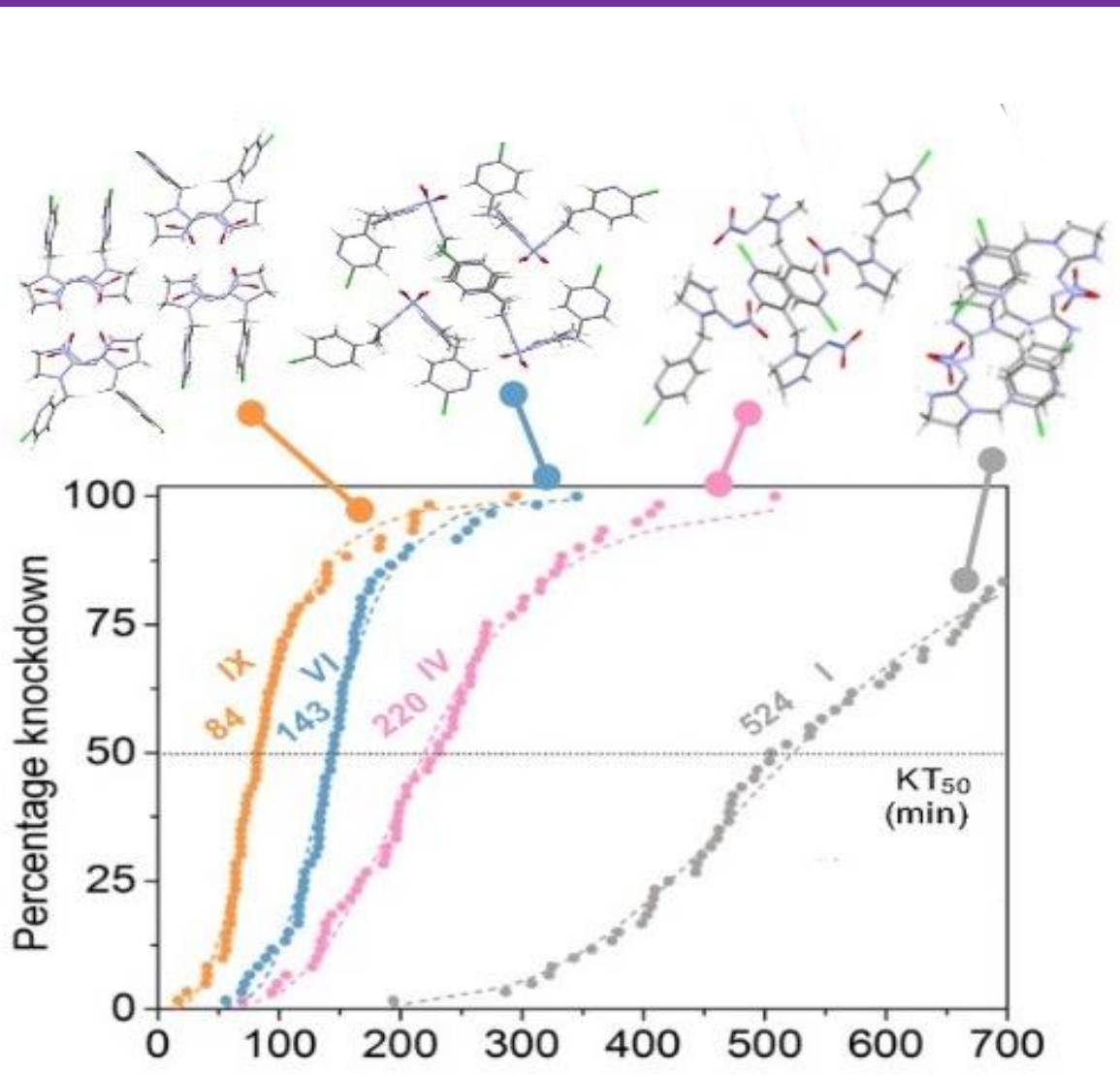


Mosquito meets polymorphous crystals

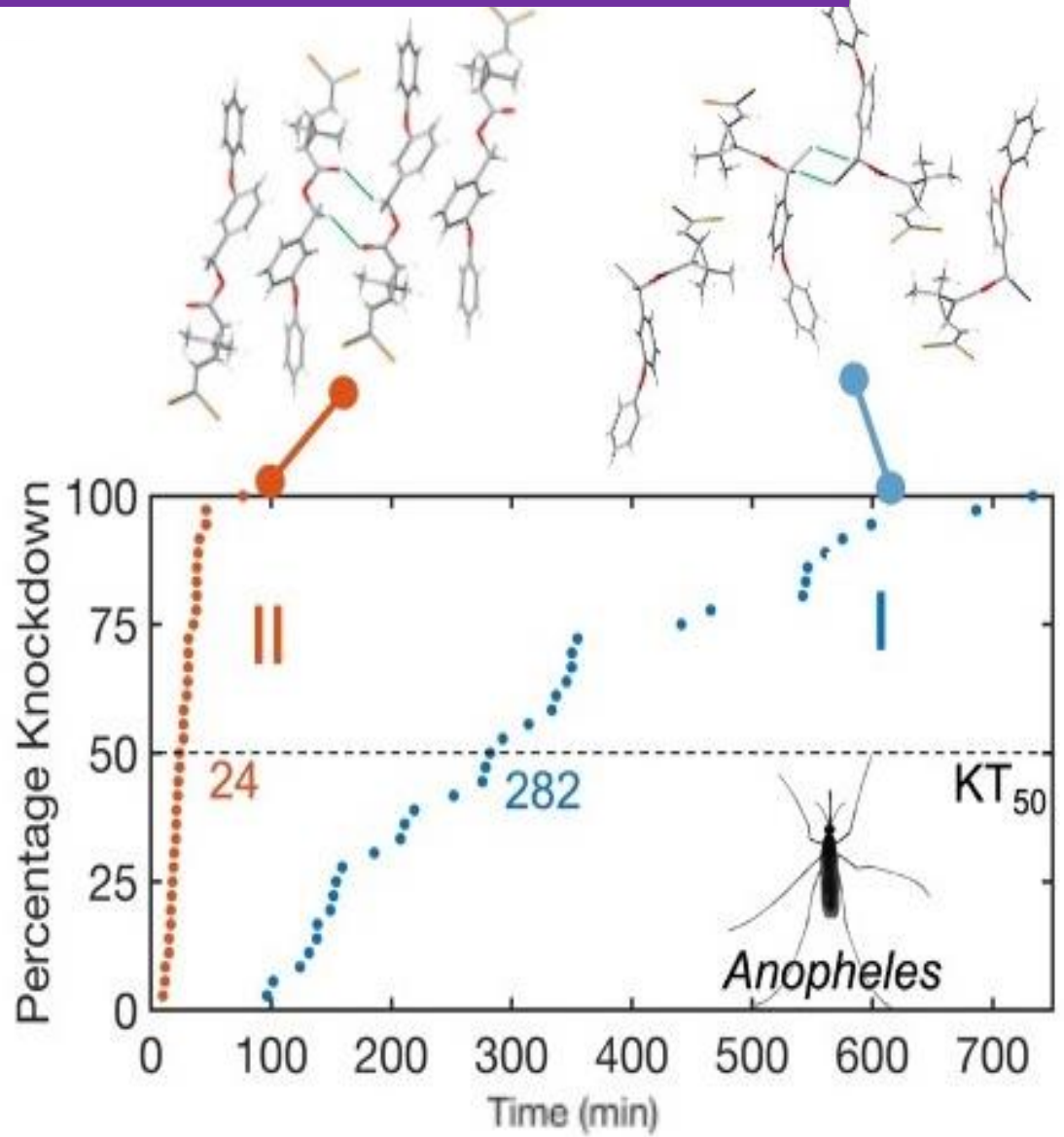




Polymorphism dependent lethality: Crystal form matters a lot



imidacloprid

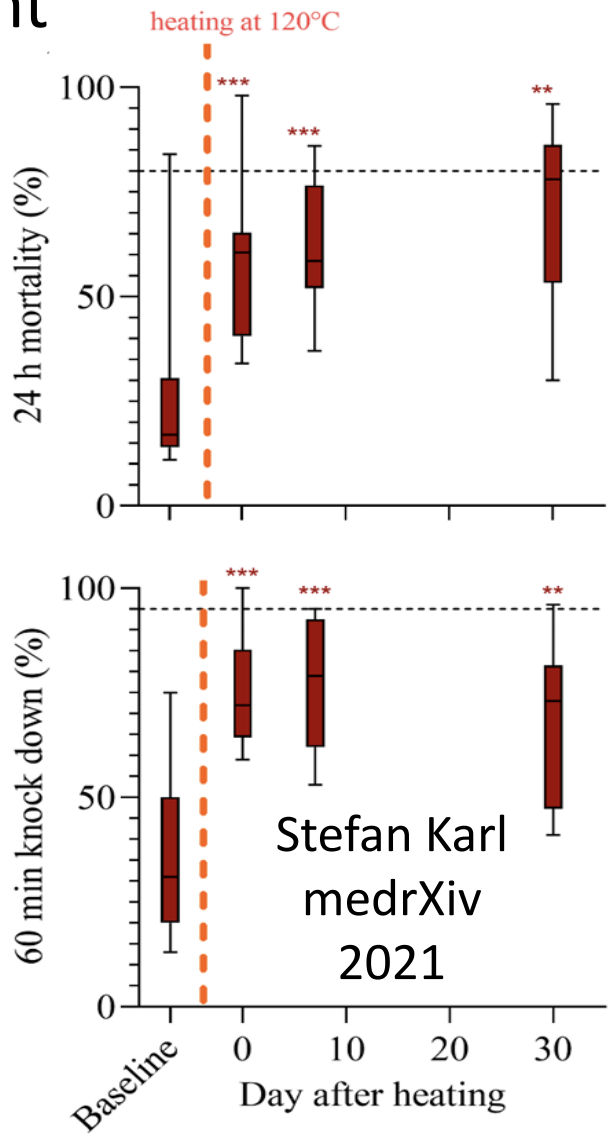


deltamethrin

Heating DeltaDust increases knockdown speed of susceptible *Anopheles* >10-fold

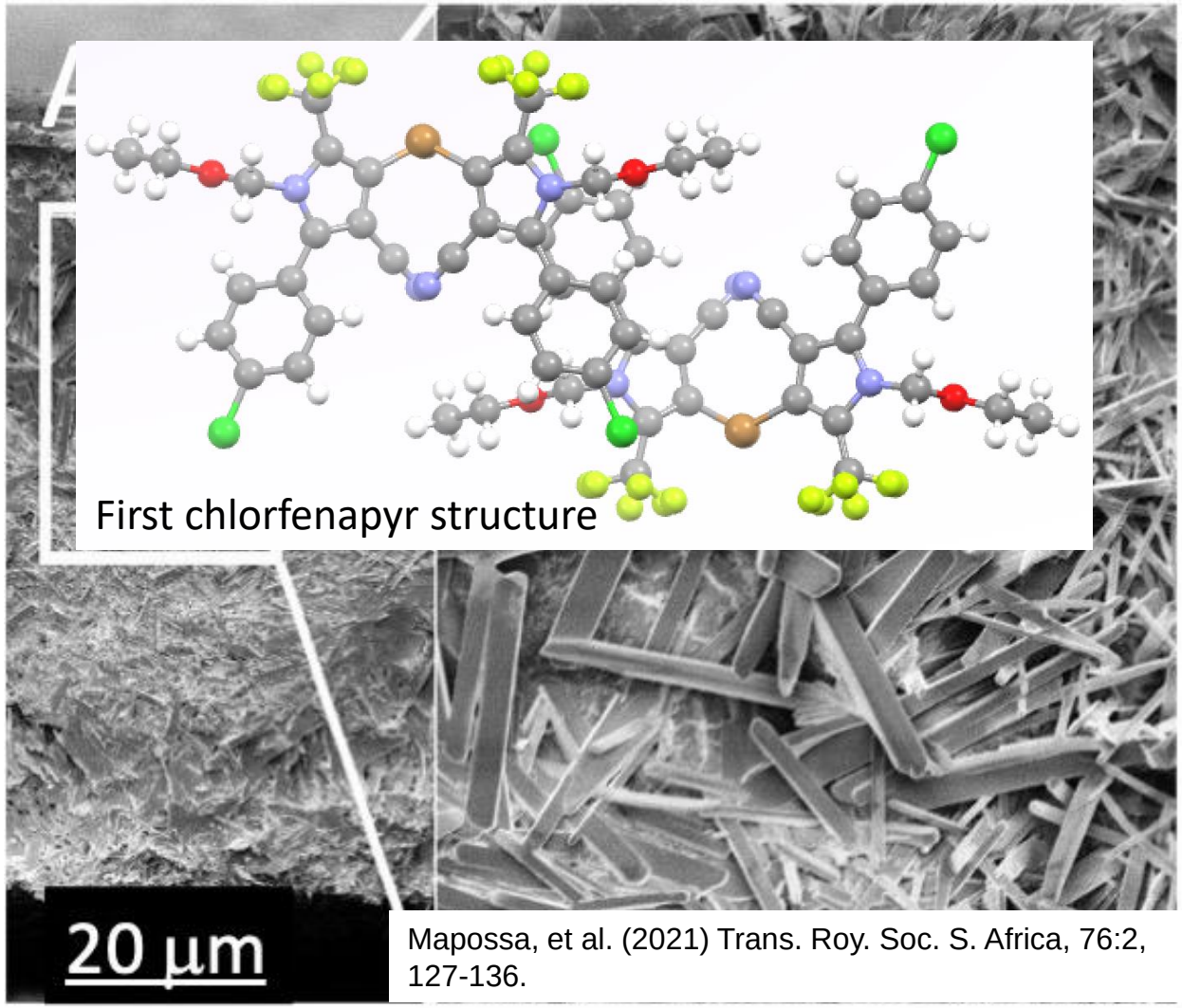
Rosemary & Jessie showed that it works for pyrethroid resistant *Anopheles*

Transformations among solid forms is consequential.



Heating ITNs shown to restore fxn.

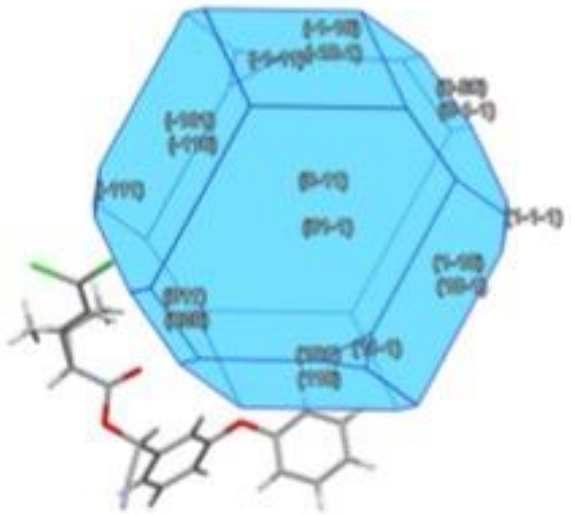
We must know which crystals grow where under what circumstances.



Mapossa, et al. (2021) Trans. Roy. Soc. S. Africa, 76:2, 127-136.

Chlorfenapyr in blown PE.

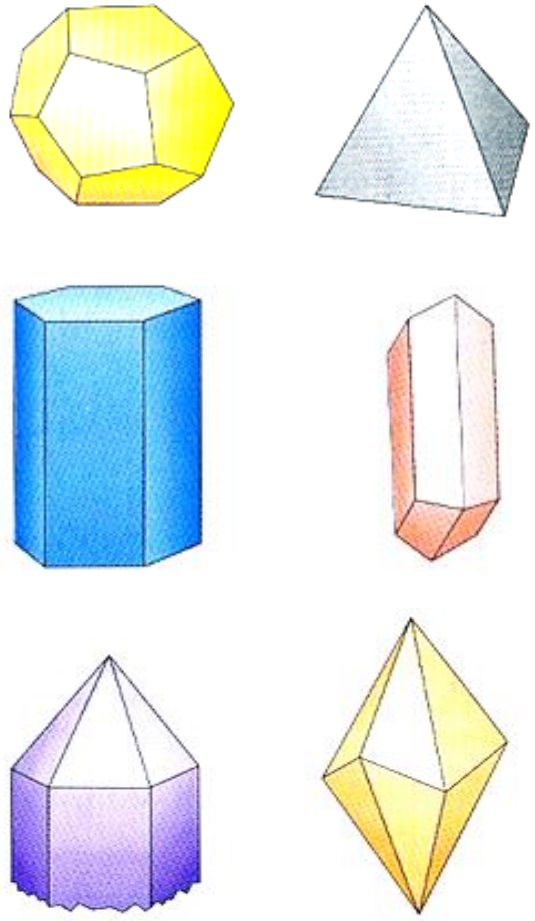
$Pna2_1$, a=11.497(2) Å, b=13.712(2) Å, and c=12.972(2) Å.

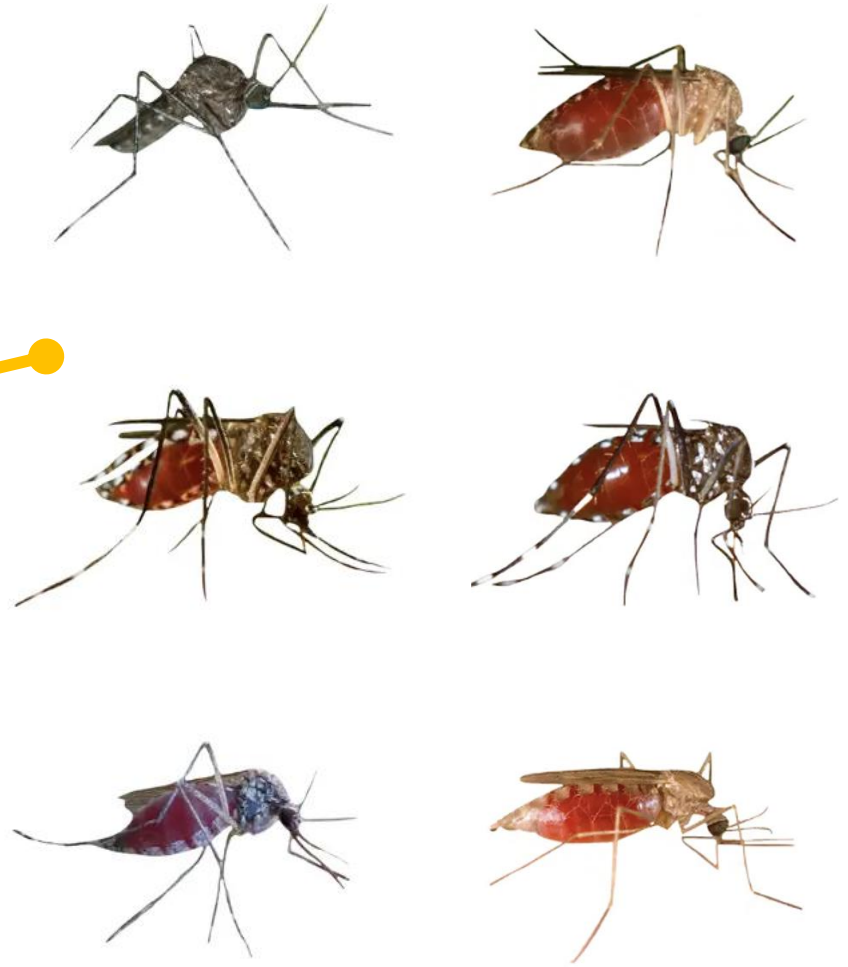


α-cypermethrin on Interceptor G1

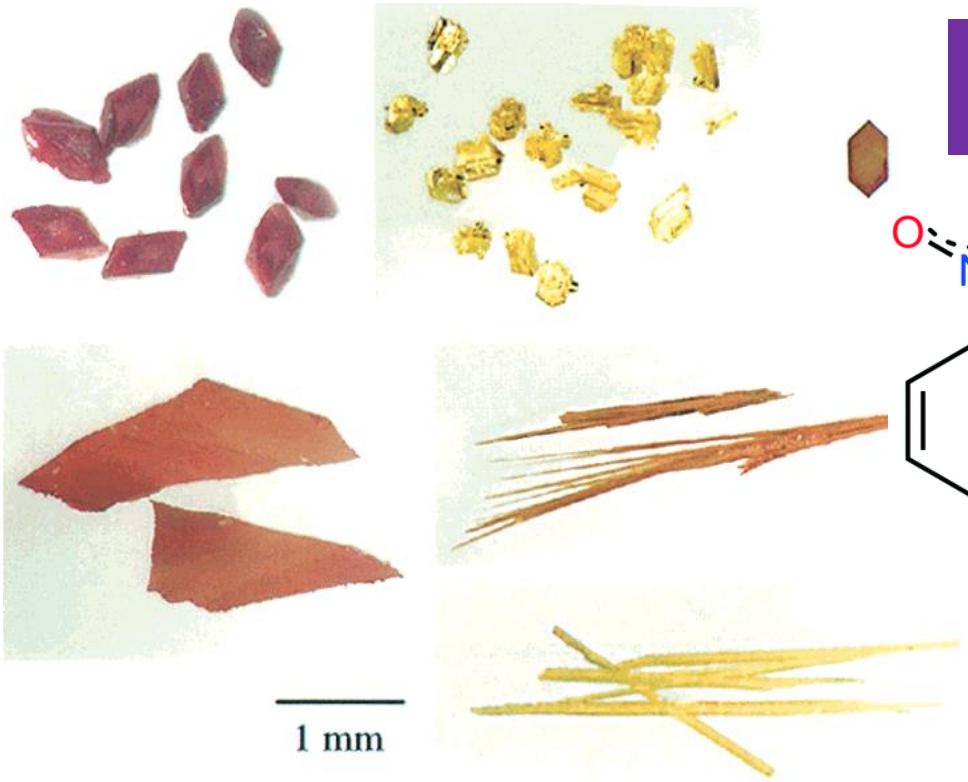
$P2_1/n$, a=11.497(2) Å, b=13.712(2) Å, and c=12.972(2) Å, β=98.349(2)°

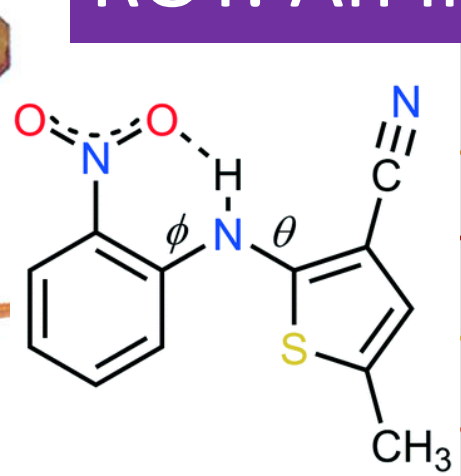
Which crystal almost as important as which mosquito.

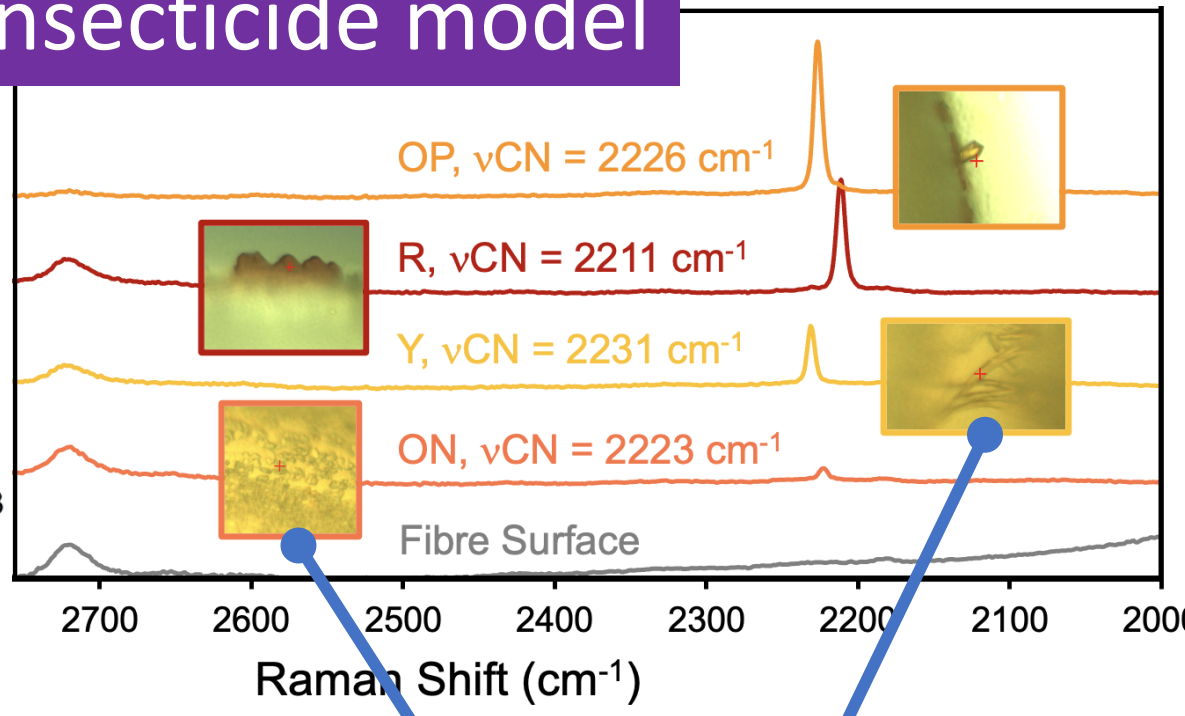




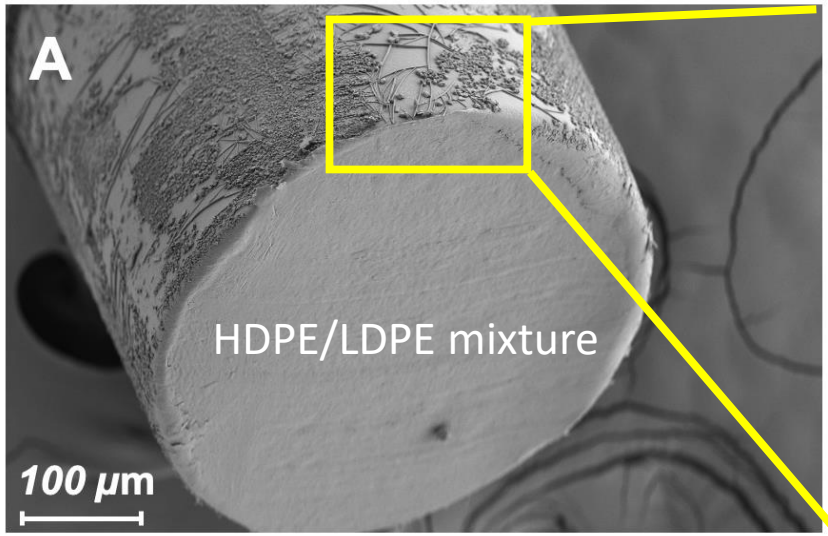
ROY: An insecticide model

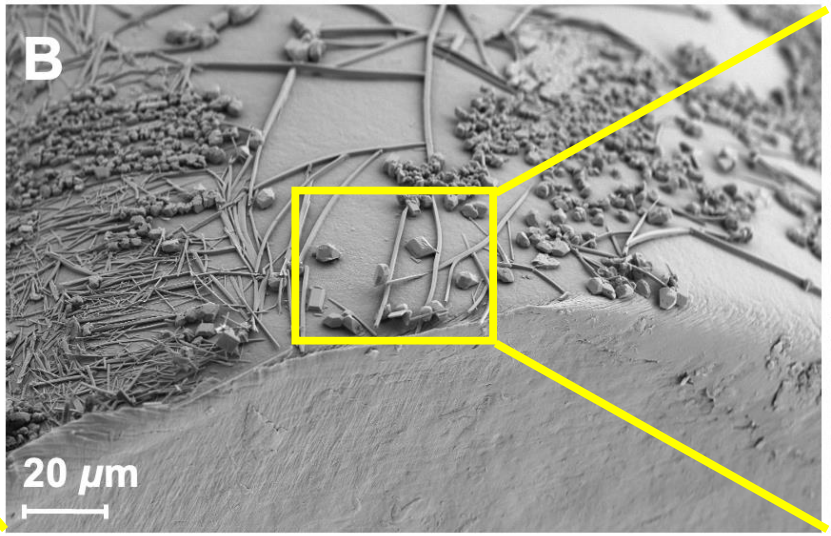


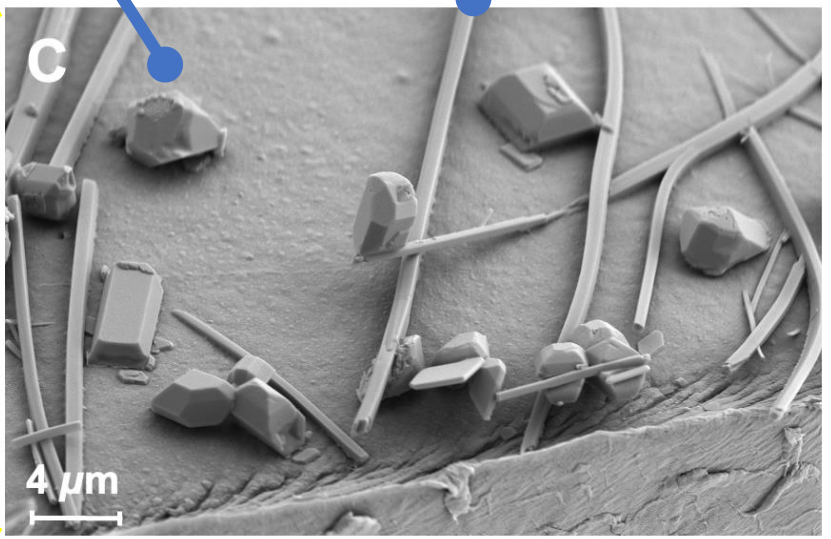




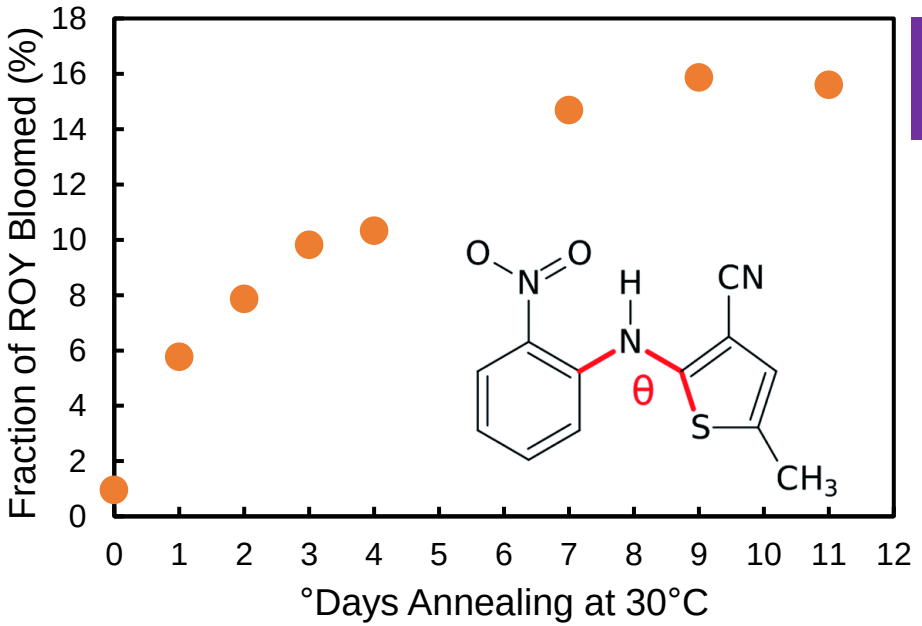
Lian Yu, 2012 (6 polymorphs). 2022 (13 polymorphs)





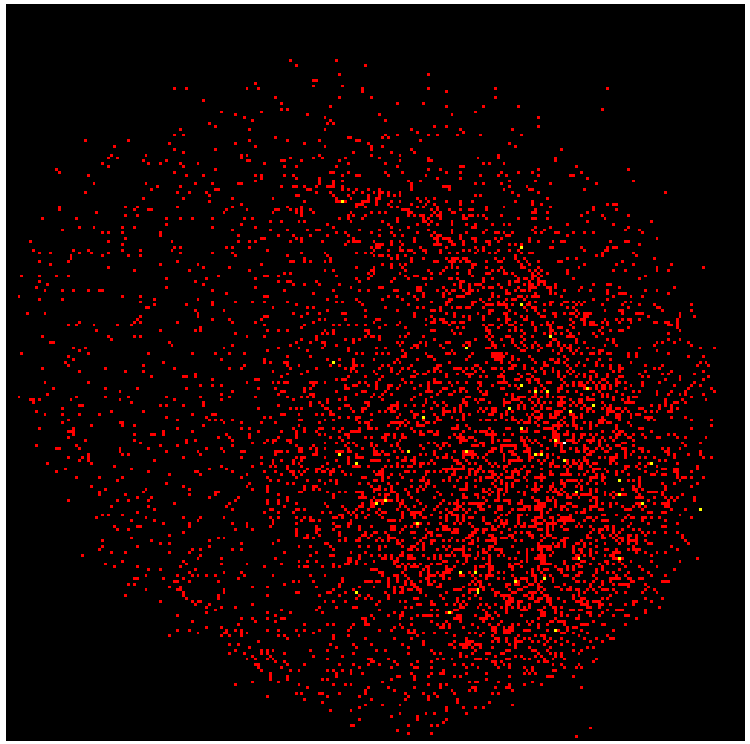


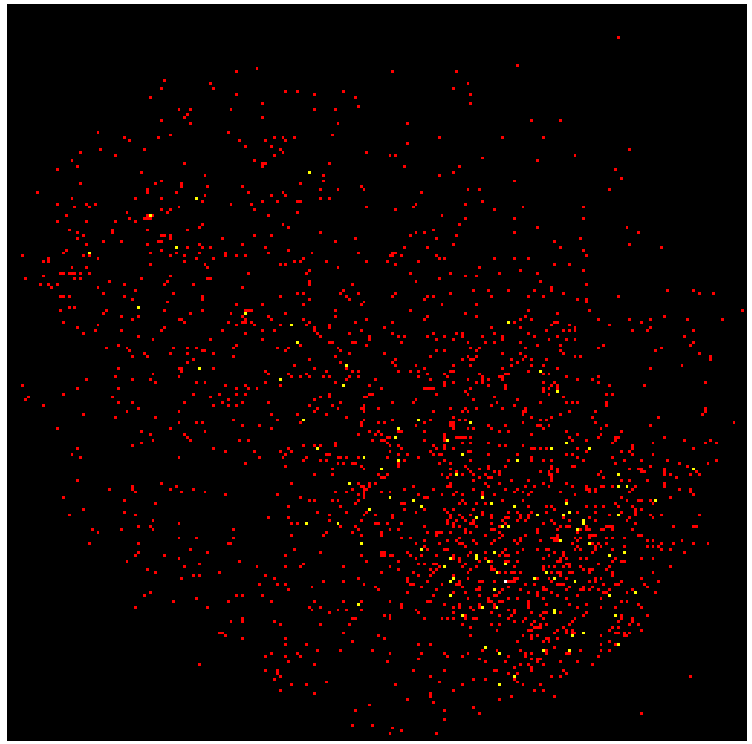
Quantification…



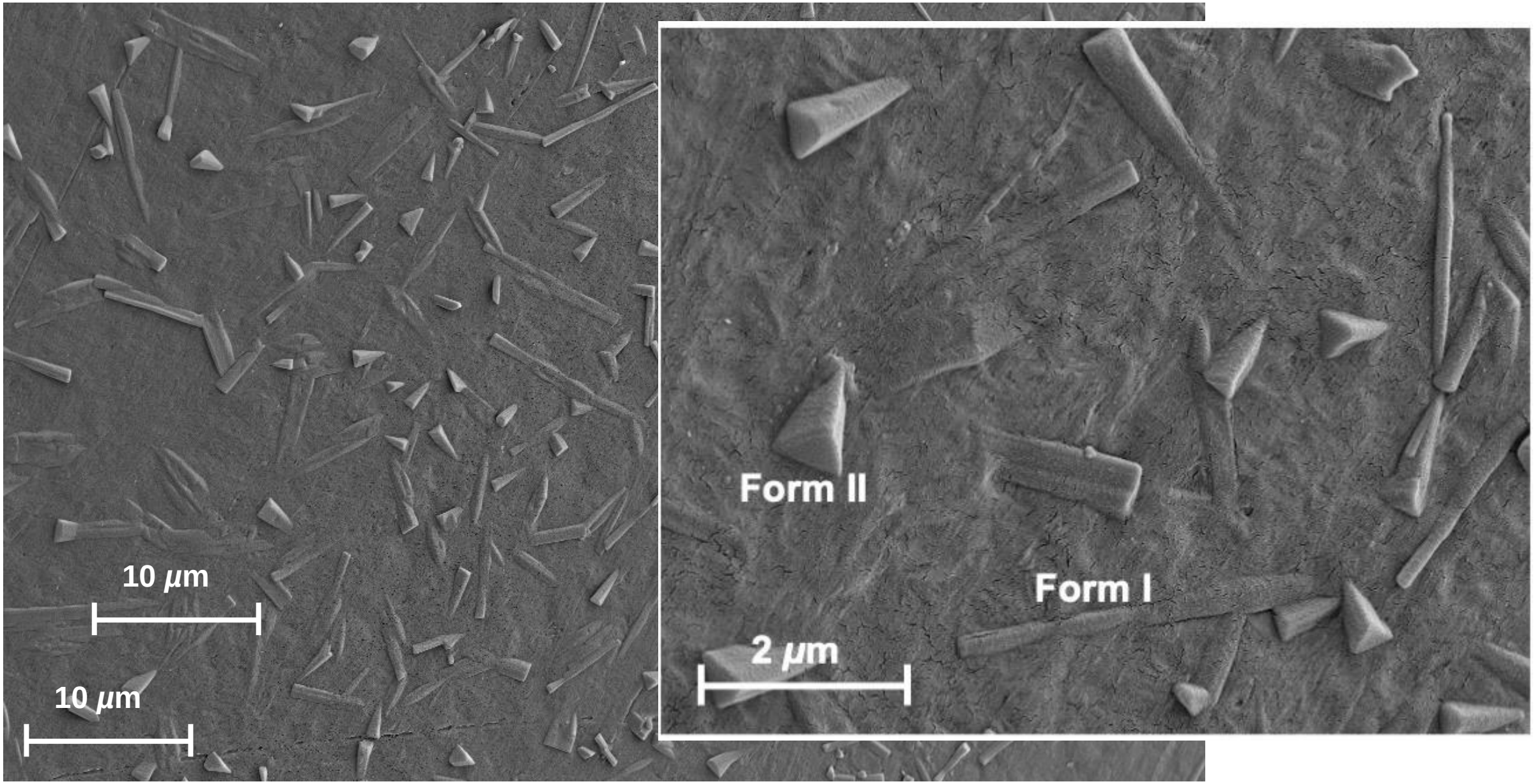
…by absorbance spectroscopy…

…or secondary ion mass spectrometry.

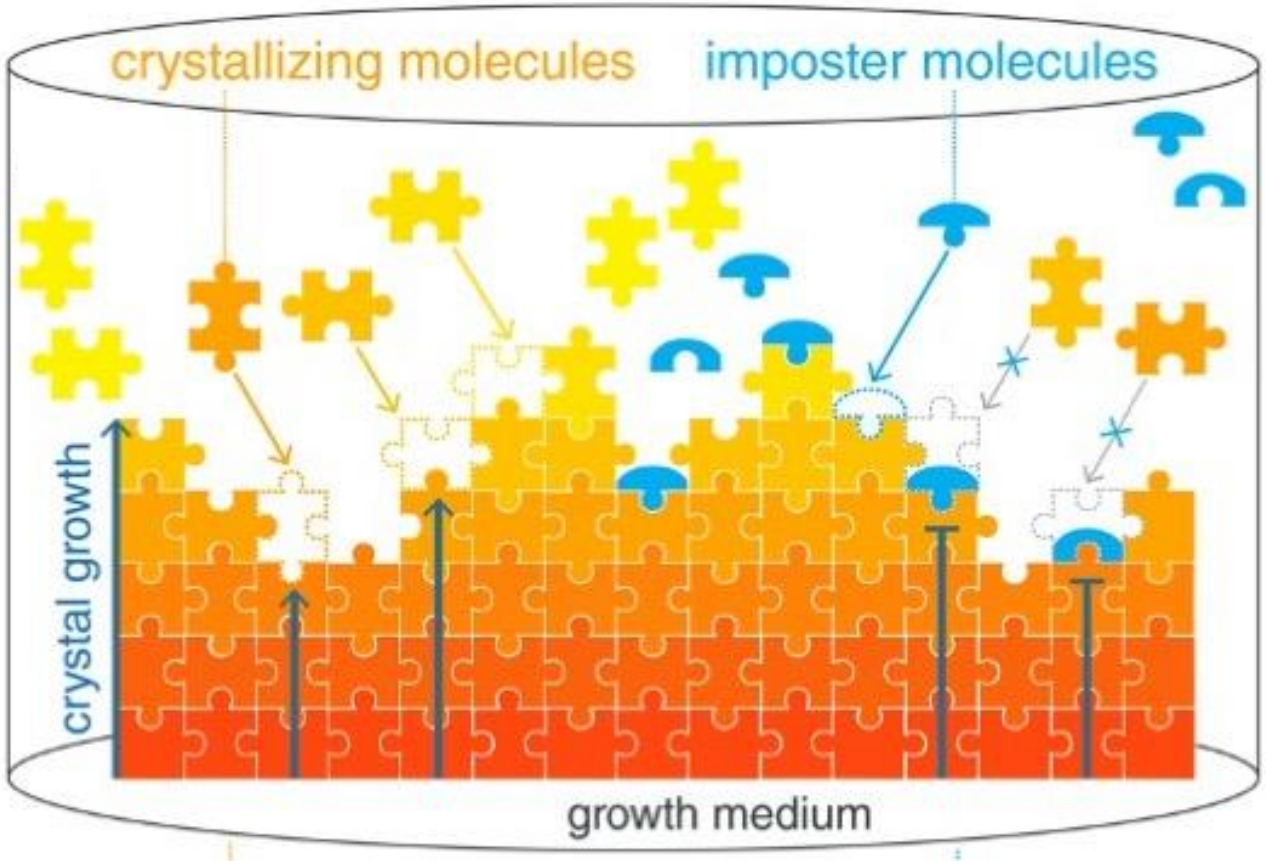


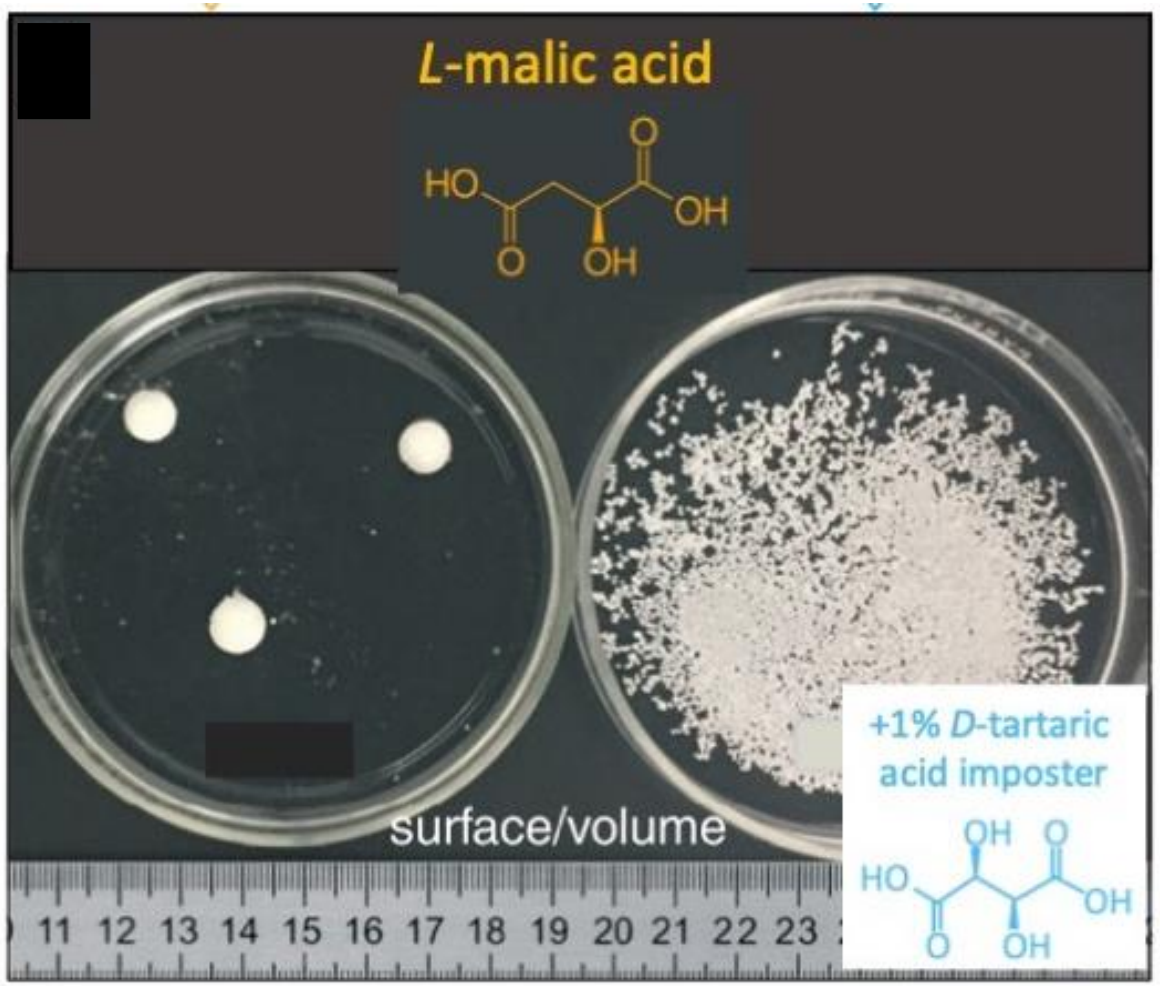


1% deltamethrin bloomed in 4 hrs from PE fiber



Tailor-made crystal growth inhibitors



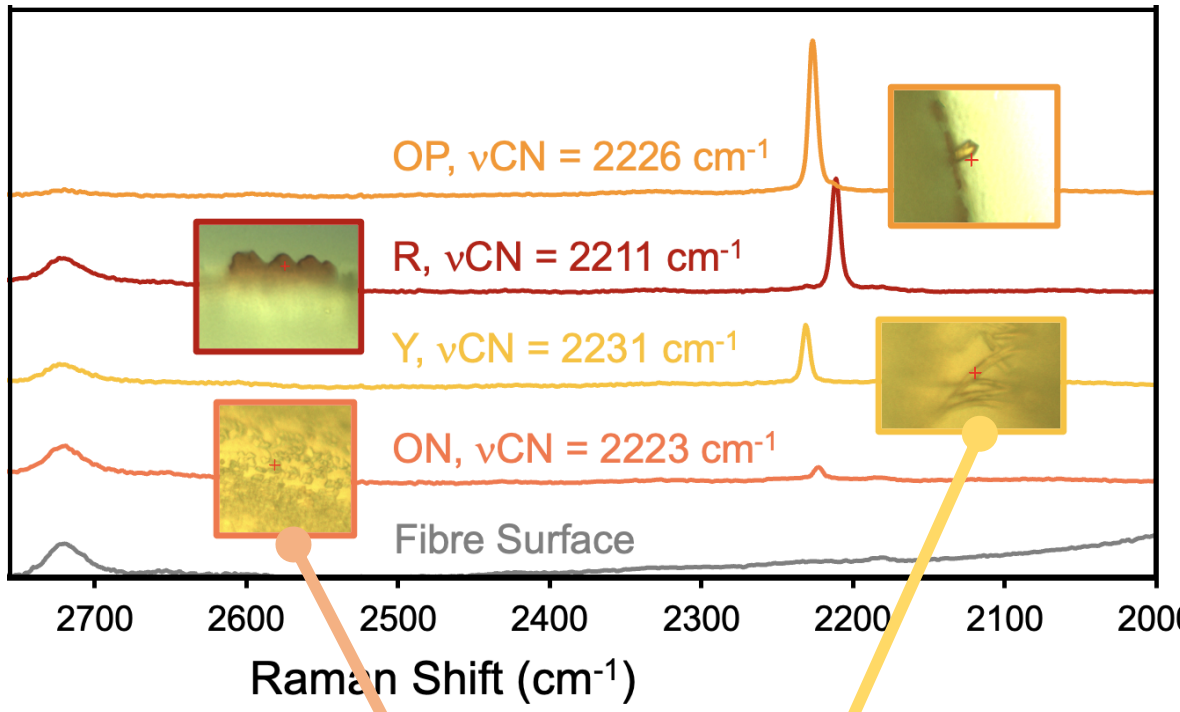


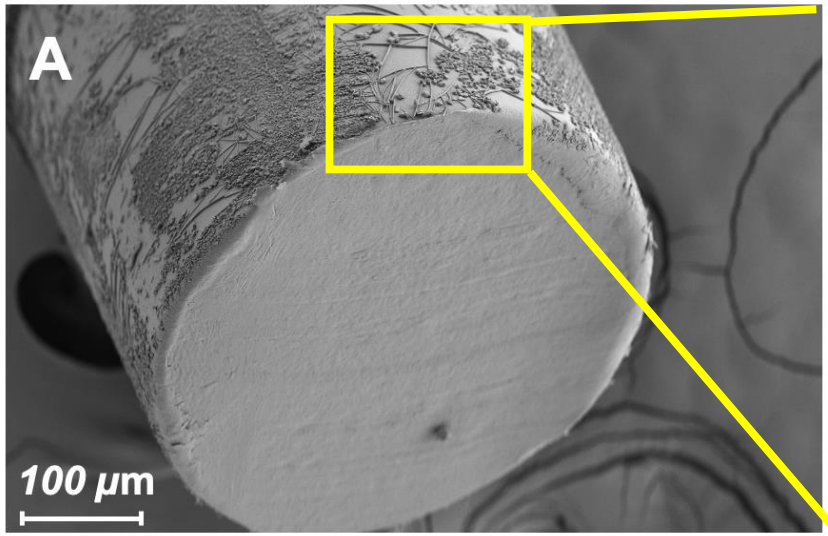
PQT/VCP Public Report

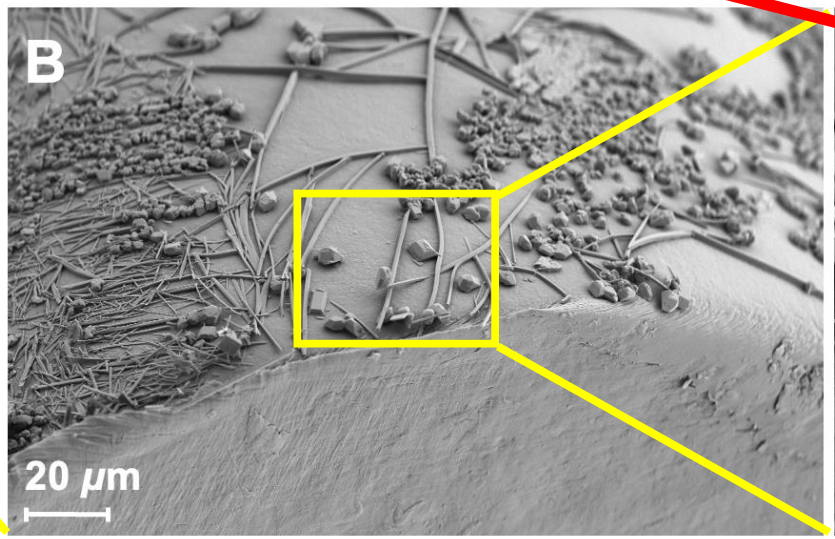
"The focus of chemistry assessments should be shifted from total AI/synergist content to surface concentration and further that which is bioavailable."

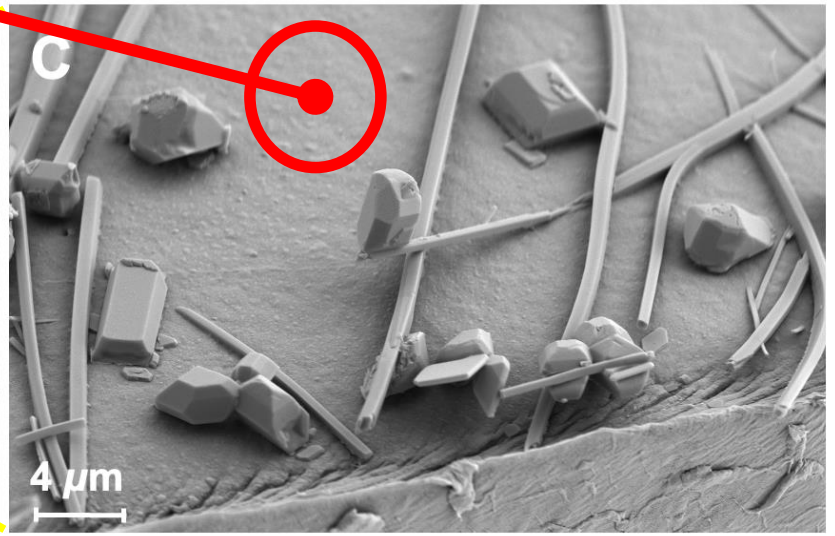
WHO

Need to amplify the signal by a factor of 10^3 or 10^6 or 10^9.

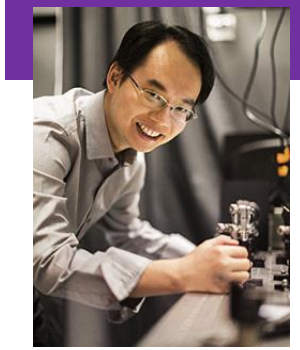




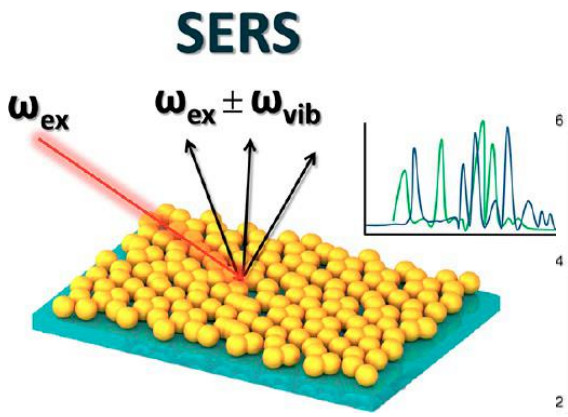


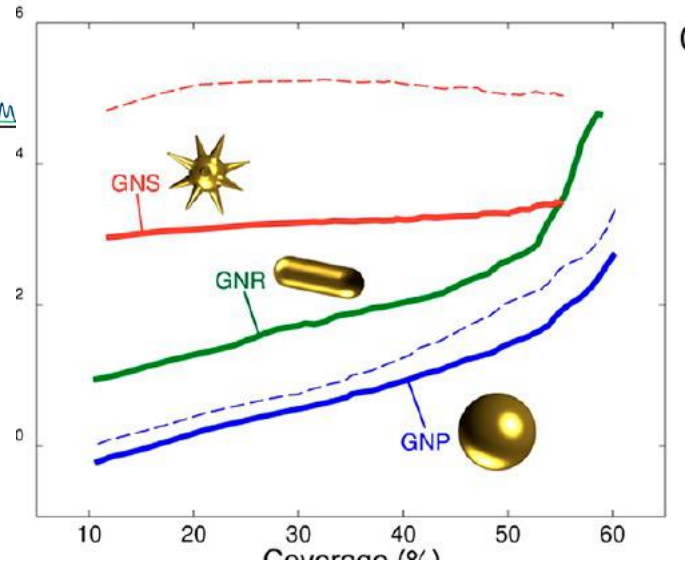


Raman enhancement techniques: Stimulated Raman scattering or surface enhanced Raman scattering or SERS (10^6 enhancements)



Professor Wei Min
Columbia U.



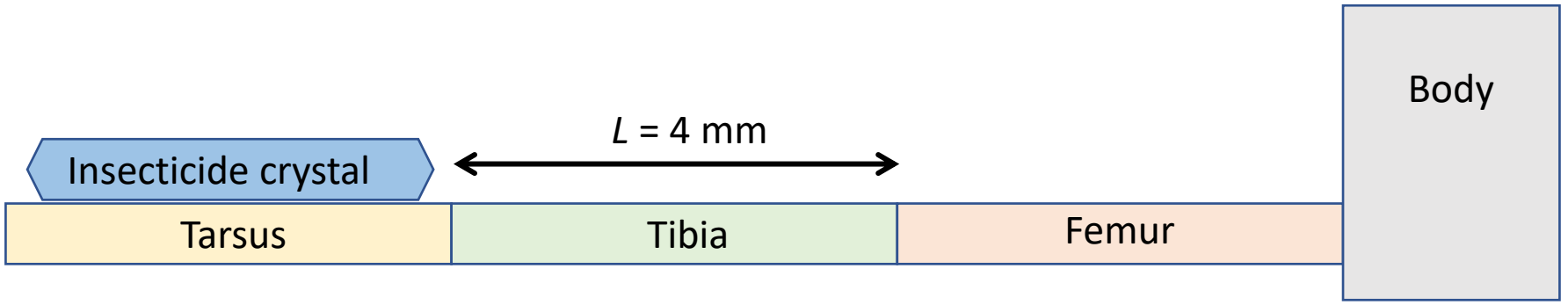


Need to build a special microscope

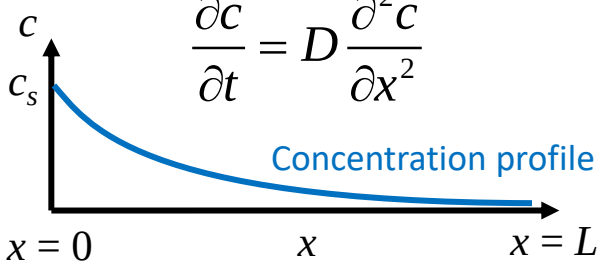
or

Prepare fibers with embedded metal nanoparticles

How fast insecticide from a dissolving crystal can get to the nervous system by diffusion through a leg?



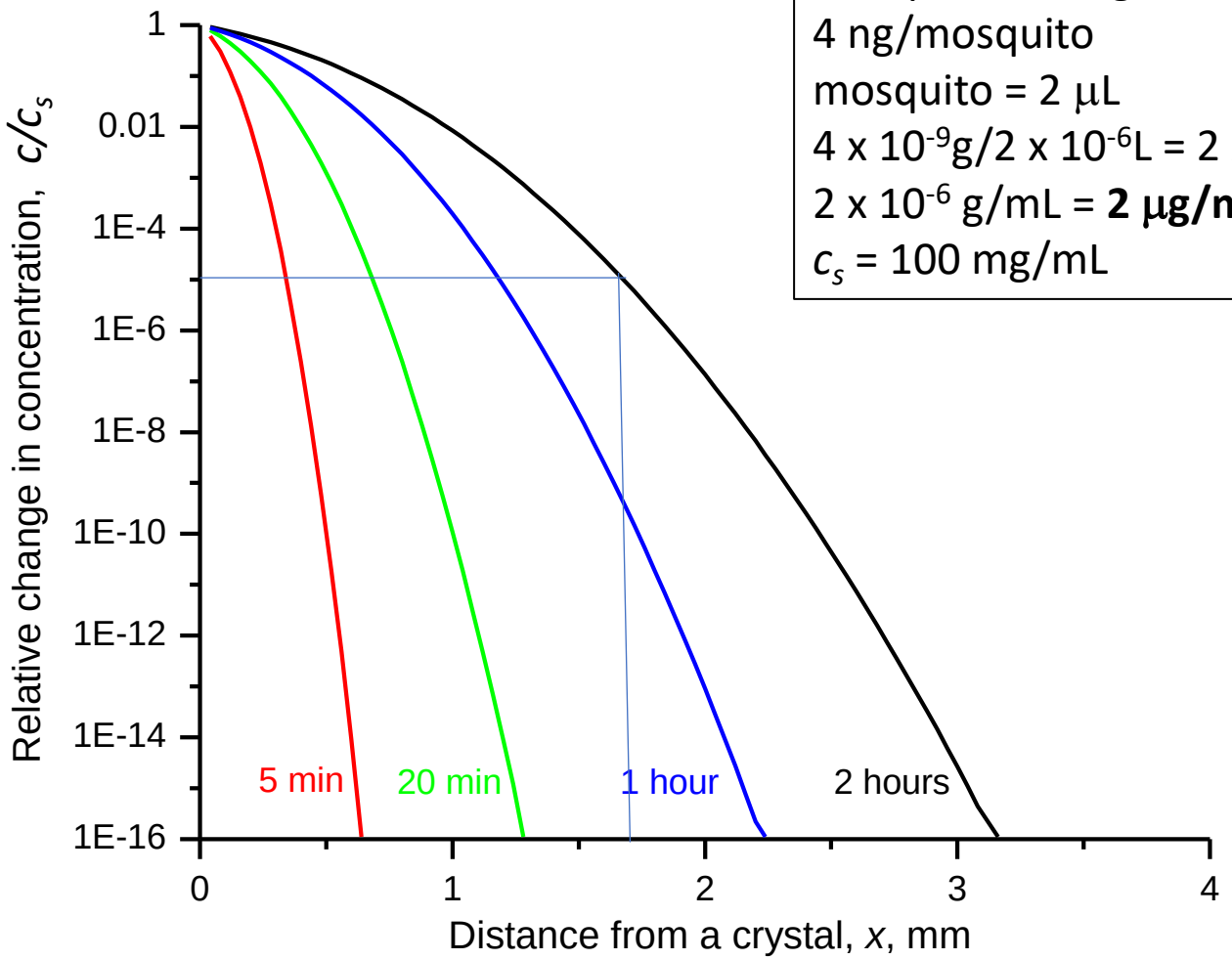
$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$



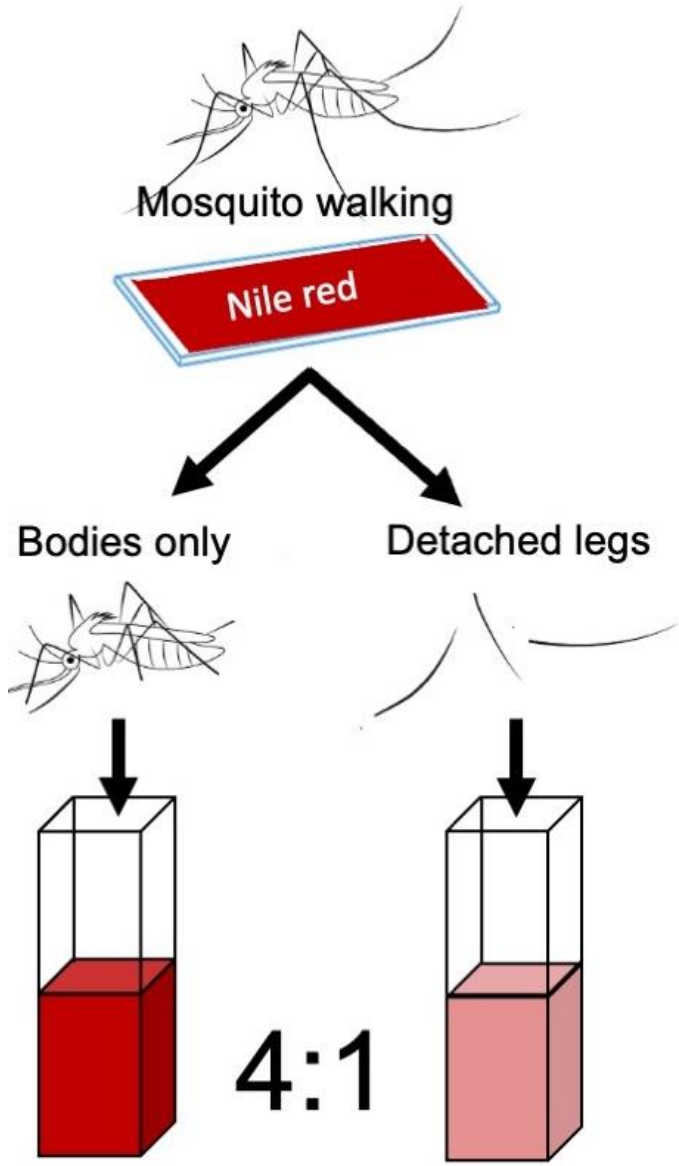
- Diffusion transport between a contact with a crystal ($x = 0$) and the beginning of femur ($x = L$); $L = 4$ mm.
- Concentration of insecticide at $x = 0$ is equal to the highest spontaneously attainable concentration of insecticide in hemolymph i.e. to its solubility c_s. This concentration is established immediately upon contact with the crystal and does not change over time $c(x=0, t) = c_s$.
- We assume $c_s = 100$ mg/mL, solubility of insecticide in organic solvents. Likely much smaller.
- Initial concentration in tibia is zero, $c(x, t=0) = 0$.
- Diffusion coefficient is assumed to be close to that for water, $D = 10^{-5}$ cm^2/s. Likely much smaller.

Mosquito legs are long. Not much gets very far very fast

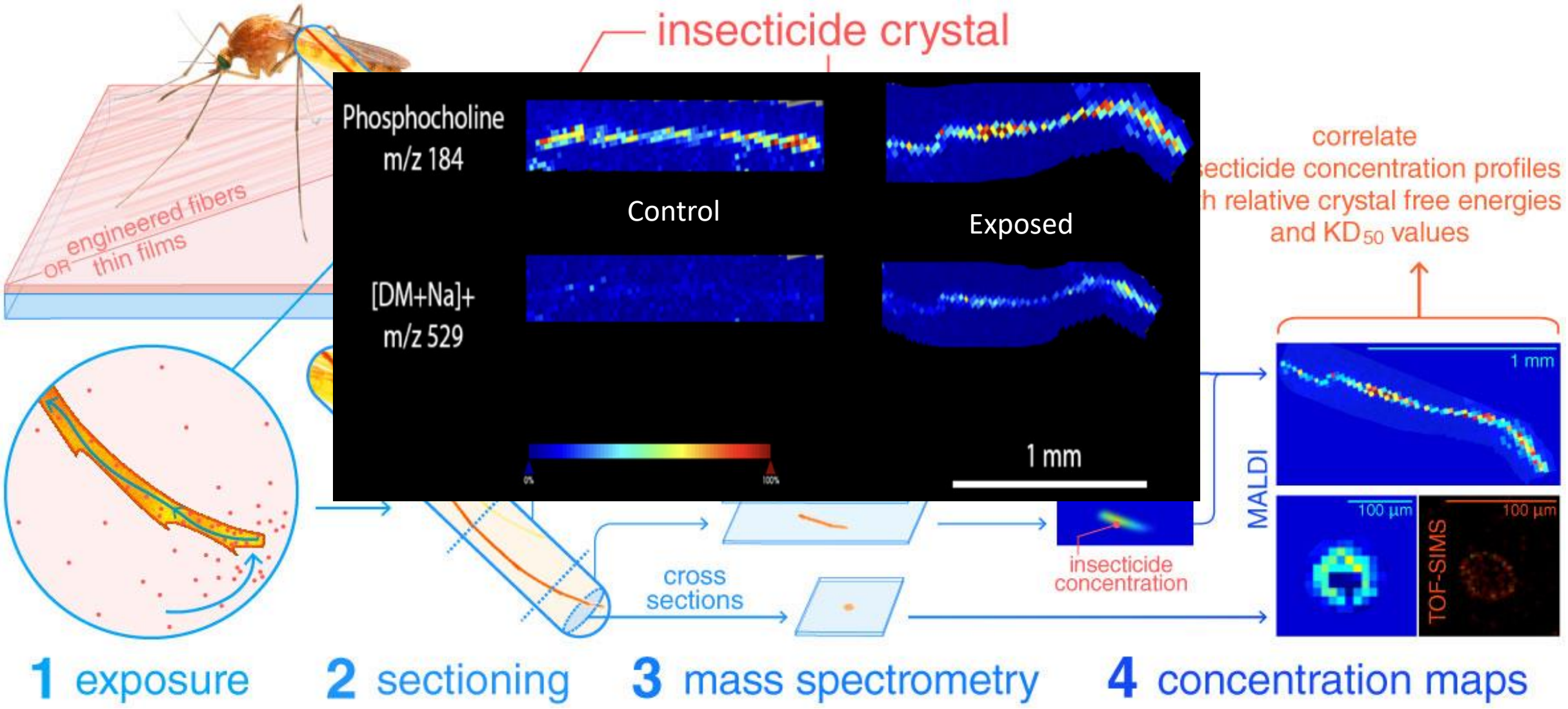
$$c(x) = c_s \left[1 - erf\left[\frac{x}{\sqrt{Dt}}\right]\right]$$



LD_{50} permethrin 2 ng/mg.
mosquito = 2 mg
4 ng/mosquito
mosquito = 2 µL
4×10^{-9}g/2×10^{-6}L = 2×10^{-3} g/L
2×10^{-6} g/mL = **2 µg/mL**
c_s = 100 mg/mL



Mass spectrometry imaging for measuring diffusion through leg



Bryan Erriah

- Prof. Michael D. Ward
- Prof. Alexander Shutkenberg
- Leilani Smith

- Dr. Jingxiang Yang (now Prof. in Nankai)
- Dr. Xiaolong Zhu (now at Merck)

- Dr. Ye He (City University of New York)
- Dr. Tai-de Li (City University of New York)

Cone bioassays for estimating bioefficacy in quality assurance

Swiss TPH
Swiss Tropical and Public Health Institute

ISO 9001:2015 certified
IFAKARA HEALTH INSTITUTE
research | training | services

Cone bioassays for estimating bioefficacy in quality assurance

Seth Irish

(on behalf of Stephen Mbwambo and colleagues)

PNGIMR

CPTU
sanas OECD G0033; 2021 accredited

What I will talk about today

The results from cone tests with susceptible mosquitoes are broadly reproducible between sites, particularly when mortality is the endpoint and at high and low mortalities

Bioassays have intrinsic variability - this needs to be considered, and this variability in bioassays may be considered rather than just putting in a hard threshold

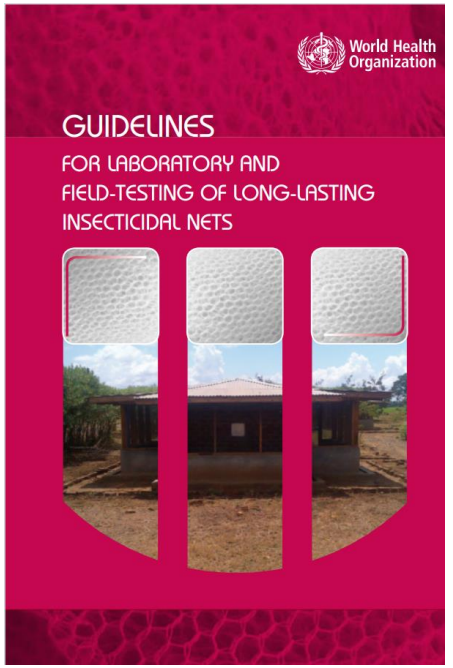
Cone tests can identify nets that are performing poorly

Mortality appears to be a more stringent endpoint and better correlated between labs

According to the literature, the large majority of nets tested prior to distribution met and exceeded the 80% mortality threshold.

Background

- Millions of ITNs being distributed each year
- A subsample of nets from each shipment are submitted for pre-distribution quality control
- While chemical content is assessed, there is no assessment of bioefficacy of the insecticide on the surface of the net
- As a result, occasionally nets have arrived in country that do not fulfil the recommended level of bioefficacy (≥ 80% mortality after 24 h or ≥ 95% knock-down 60 min after exposure)
- While cone tests are routinely used for assessment of ITNs in the field, there is some reluctance to use them for quality control, due to variation in the assay
- However, until there is a standardized way of assessing bioefficacy by other means, the cone test may be the best option.



Aim of this work:

- Determine whether cone bioassays can provide reproducible results when the same nets are tested in two laboratories
- Review relevant literature

Methods

4x PermaNet 2.0 (2019)

4x PermaNet 2.0 (2012)

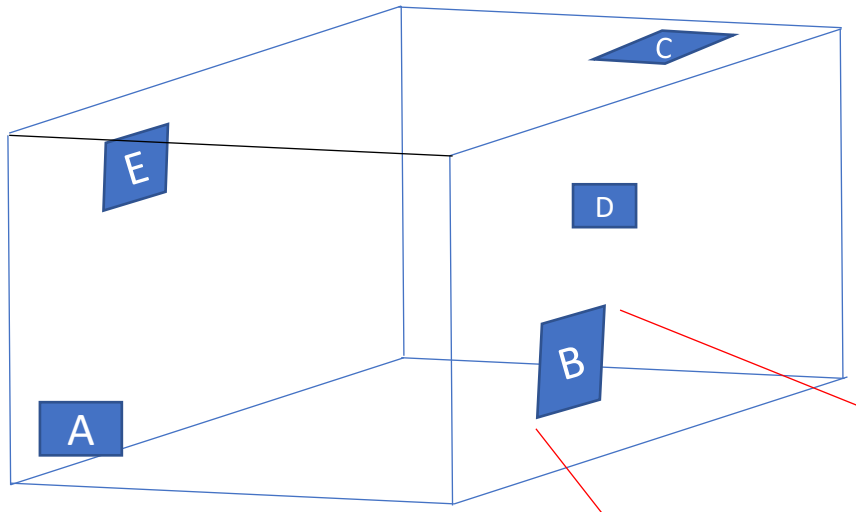
4x Interceptor (2020)

4x SafeNet (2019-2020)

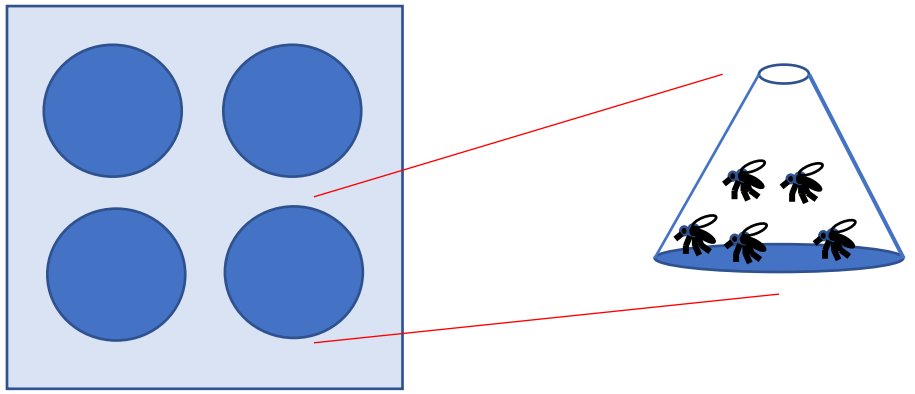
4x Yorkool (2019)

SafiNet (TZ)
Baomei (PNG)
Control

For each net, 5 samples were cut



For each net piece, 4 cones were used (5 mosquitoes per cone)



5 net brands/years	4 nets of each brand	5 samples taken from each net	4 cones used per net sample	5 mosquitoes tested per net sample
5 net brands	20 nets	100 net samples	400 cone tests	2000 mosquitoes

Methods

	Bioassay test in IHI	Cone bioassay test in PNGIMR
Number of ITNs tested	20 nets (100 net pieces)	20 nets (100 net pieces)
Mosquitoes exposed	20 per net piece (cone bioassay) 100 per net piece (tunnel tests)	20 per net piece (cone bioassay)
Experiment conditions	27±1°C (cone bioassay) 55% - 82% RH (cone bioassay) 27ºC ± 2 ºC (tunnel tests) 60% - 100% RH (tunnel tests)	28 ±4°C (cone bioassay) 53% - 71% RH (cone bioassay)
Mosquito species	Pyrethroid susceptible *An. gambiae* s.s	Pyrethroid susceptible *An. farauti* s.s
Mosquito age	3-5 days (cone bioassay) 5-8 days (tunnel tests)	2-5 days (cone bioassay)
WHO efficacy criteria	≥ 95%KD60 or ≥80% M24 (cone bioassay) ≥ 90% BFI and/or ≥80% M24 (tunnel tests)	≥ 95% KD60 or ≥80% M24 (cone bioassay)


IHI
PNGIMR

Methods

- Literature review
  - conducted to look at all cone bioassays on pyrethroid-treated nets in countries pre-distribution

- Statistical analysis
  - Evaluation of knockdown and mortality using standard WHO thresholds
  - Spearman rank correlation coefficient to look at correlation
  - Bland-Altman methods to assess agreement between the measurements of the two sites
  - Cohen's kappa to evaluate pass/fail results

Results

- At PNGIMR, **8/20** nets met initial cone test criteria (≥95% knockdown or ≥80% mortality)
- At IHI, **13/20** nets met initial cone test criteria, but all nets not passing the cone test did meet the tunnel test criteria (≥90% blood feeding inhibition or ≥80% mortality)

Results

- The relationship between knockdown and mortality was examined:
  - IHI: R=0.36, showing low correlation of KD and mortality
  - PNGIMR: R=0.78, showing higher correlation of KD and mortality

Results

- Correlation between IHI and PNGIMR results
  - Knockdown (R=0.6, p=0.002)
  - Mortality (R=0.9, p<0.001)

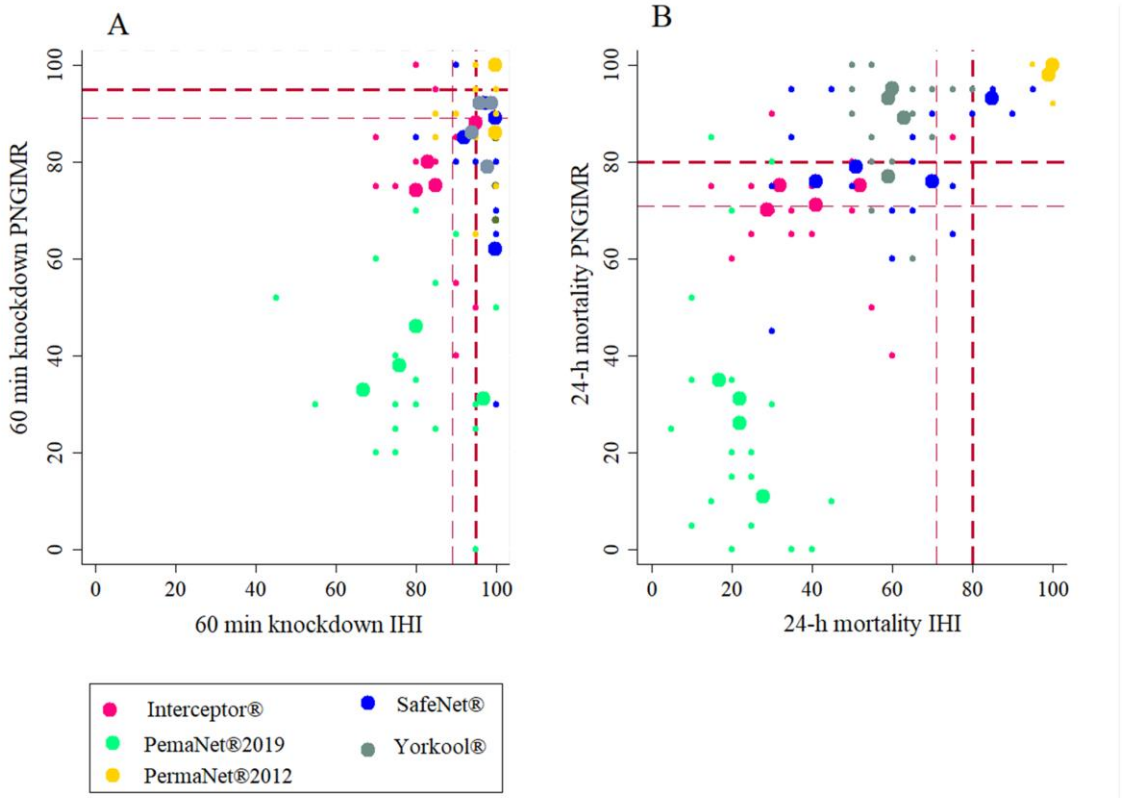
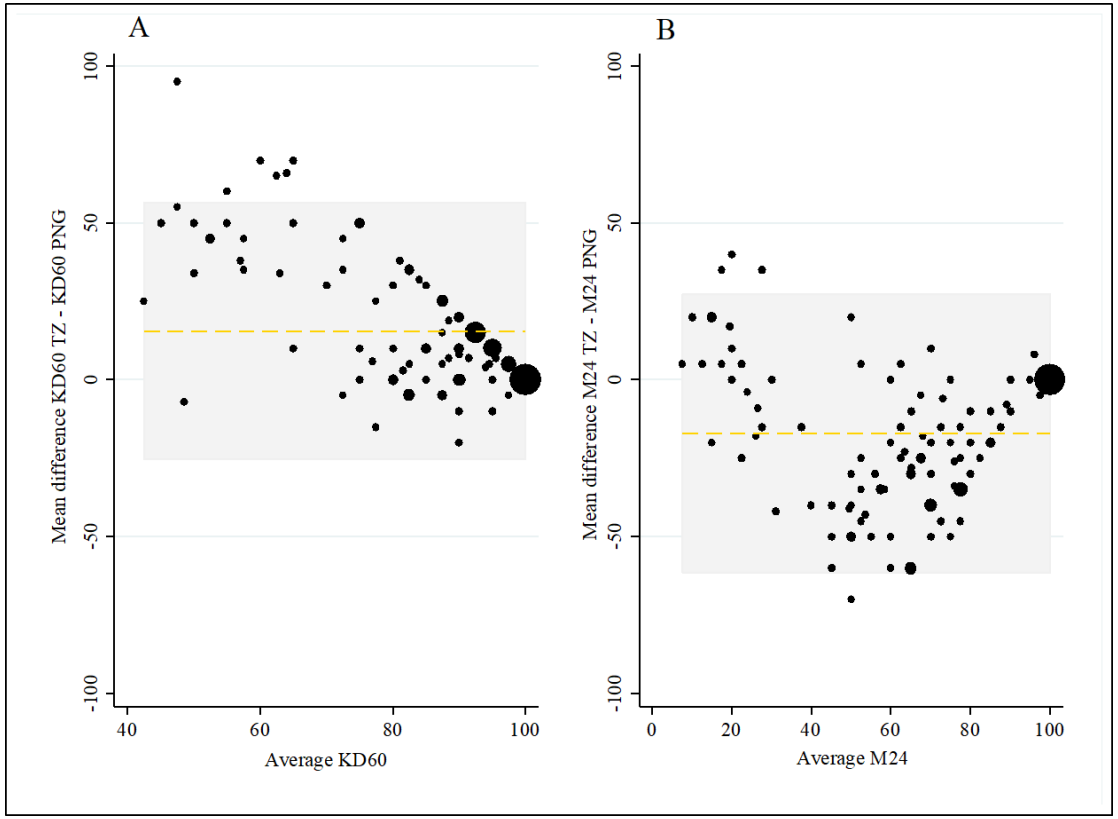


Figure 3 Correlation of cone bioassay tests results between IHI and PNGIMR testing facilities. Dash line is the WHO threshold 95% KD60 (A) and 80% M24 (B). Large dots represent averages per sampled nets (4 per net type) and small dots represent all subsamples (5 per net).

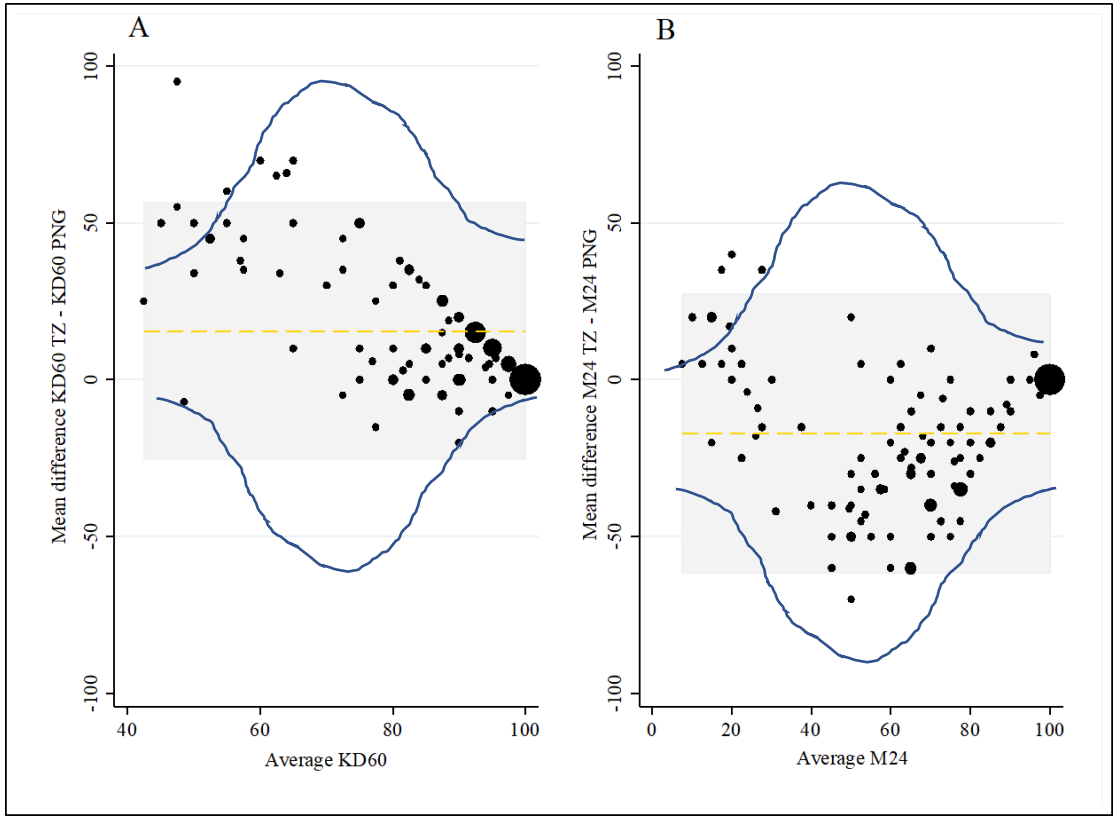
Results

- Bias (difference between 0 and the mean of the differences- IHI vs PNGIMR) was 15.5 and -17%
- The confidence intervals were -25 to 57% for knockdown and -61 to 27% for mortality. No acceptable range was predefined.
- Less than 5% of the measurements were outside the 95% CI indicating agreement, but is this agreement acceptable?



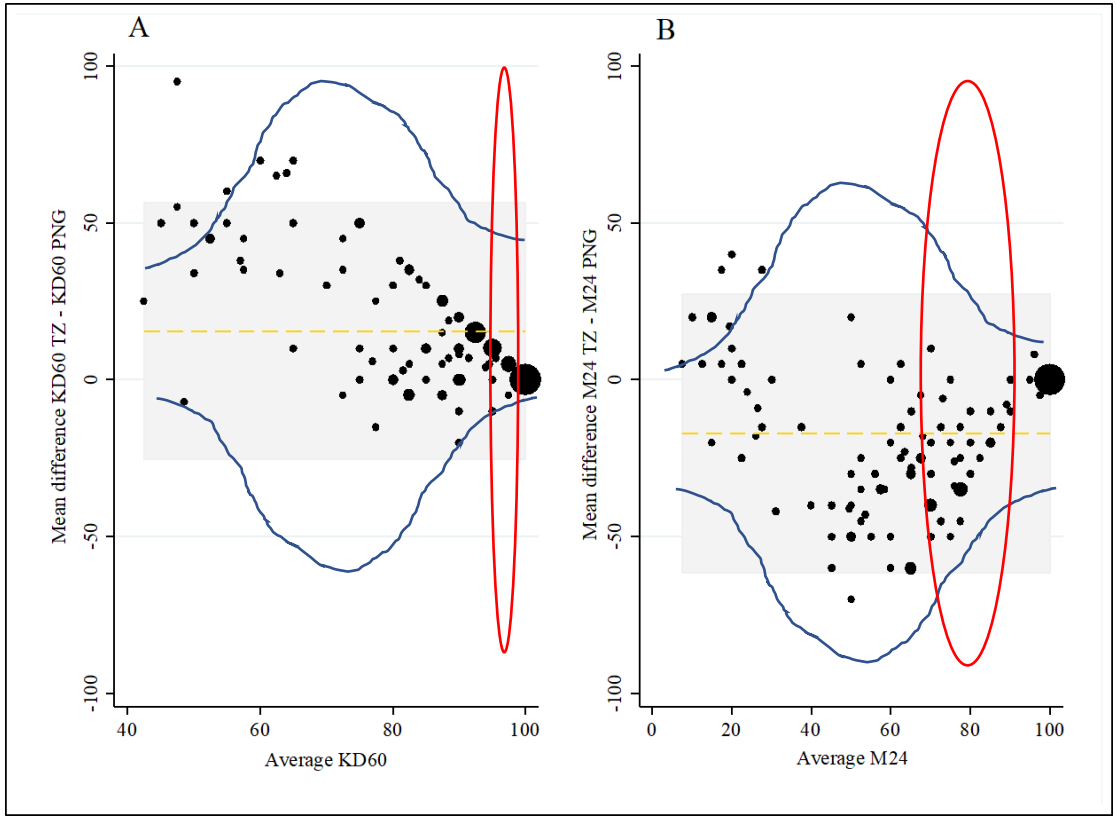
Results

- Bias (difference between 0 and the mean of the differences- IHI vs PNGIMR) was 15.5 and -17%
- The confidence intervals were -25 to 57% for knockdown and -61 to 27% for mortality. No acceptable range was predefined.
- Less than 5% of the measurements were outside the 95% CI indicating agreement, but is this agreement acceptable?



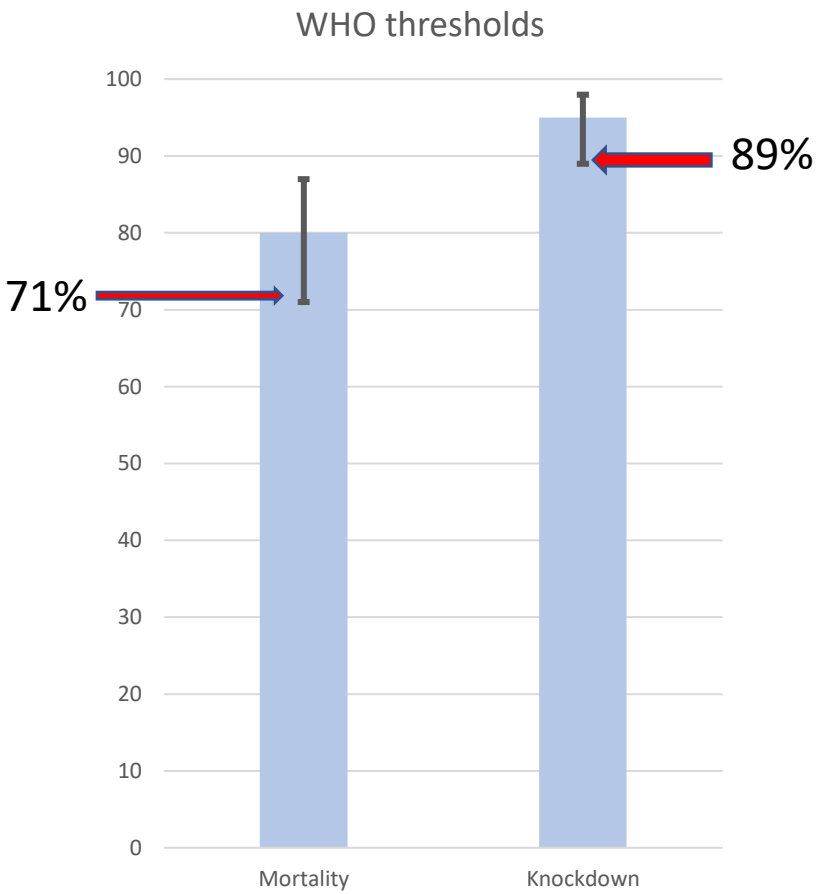
Results

- Bias (difference between 0 and the mean of the differences- IHI vs PNGIMR) was 15.5 and -17%
- The confidence intervals were -25 to 57% for knockdown and -61 to 27% for mortality. No acceptable range was predefined.
- Less than 5% of the measurements were outside the 95% CI indicating agreement, but is this agreement acceptable?



Results

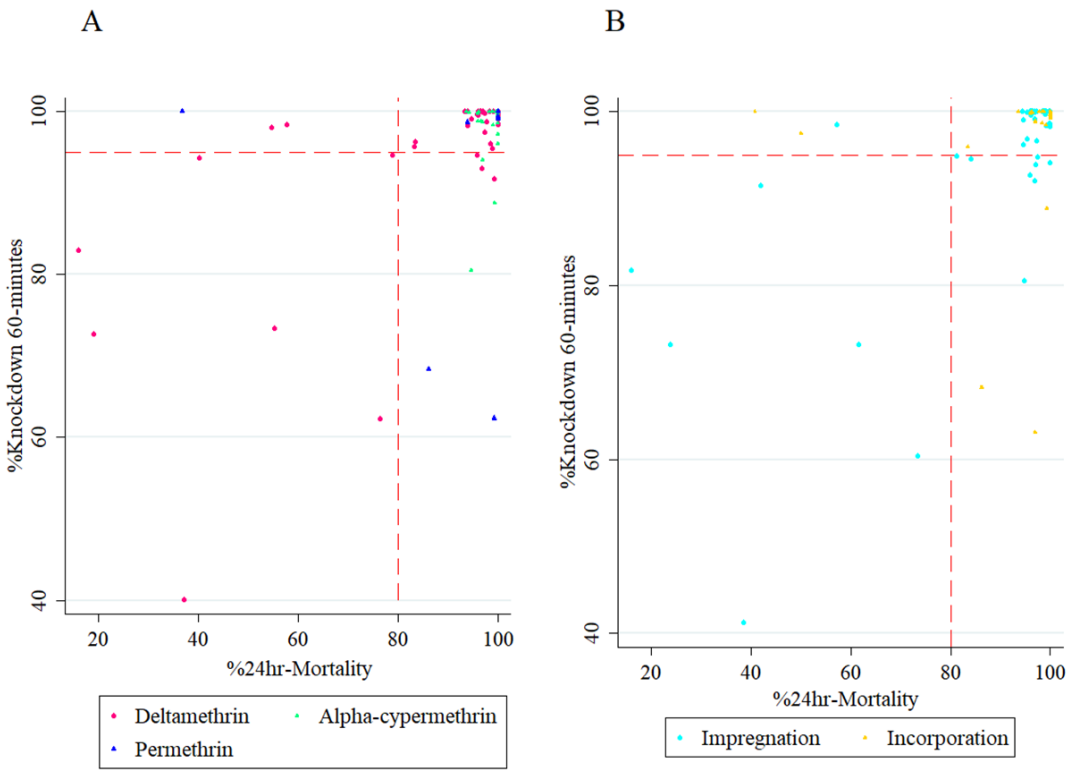
- When confidence intervals were set to the WHO thresholds, the IHI and PNGIMR results resulted in the same “pass” or “fail” determination for 90% of the nets.
- Agreement in determination was good K=0.79 (0.53-1.0)



Results

Literature review

- Majority of pre-distribution pyrethroid-treated nets had high knockdown and mortality (mean KD: 96%, mean mortality 92%)
- Out of 83 unwashed ITN results reported, only 12 reported knockdown of less than 95%, and only 8 reported mortality less than 80%



Discussion

- There are important considerations when thinking about pre-distribution quality control
  - Chemical content vs bioefficacy
  - Storage conditions
  - Variability inherent in cone bioassay (due to mosquito rearing, test procedures, etc).
  - **Laboratory differences**
- The finding of nets that succeeded in the pre-shipment assays but failed in bioassays indicate that some pre-distribution testing may be necessary
- While not perfect, cone bioassays appear to be a viable method for assessment of ITNs.

MERCI. ASANTE SANA. THANK YOU

- VCPTU team at IHI
- PNG National Malaria Control Programme
- Leo Makita
- Rotarians against malaria PNG

VCPTU
sanas OECD G0033; 2021 accredited

ISO 9001:2015 certified
IFAKARA HEALTH INSTITUTE
research | training | services

Swiss TPH
Swiss Tropical and Public Health Institute

PNGIMR

Research Article

Cone Bioassays Provide Reproducible Bioefficacy Estimates with Different Anopheline Mosquitoes and Can Be Used for Quality Assurance of Pyrethroid Insecticide Treated Nets

Stephen Gabriel Mbwambo[1] Email
Nakei Bubun[2]
Emmanuel Mbuba[1]
Jason Moore[1]
Kasiani Mbina[1]
Dismas Kamande[1]
Moses Laman[2]
Emmanuel Mpolya[3]
Olukayode Odufuwa[1]
Tim Freeman[4]
Stephan Karl[2]
Sarah J Moore[1] ORCID

On the surface: Two simple chemical methods to determine the bioavailable surface concentration of insecticide for insecticide treated net (ITN) evaluation.

On the surface: Two simple chemical methods to determine the bioavailable surface concentration of insecticide for insecticide treated net (ITN) evaluation.

Ole Skovmand,

D M Dang, T Q Tran, R Bossellman, SJ Moore

What I will talk about today

- Quantification of AI or synergist on the surface of the fibres using two chemical methods
  1. SWAM – **S**urface **W**ash **A**nalytical **M**ethod
  2. BAM – **B**efore and **A**fter **M**ethod

- Relationship between chemical methods and bioefficacy measured by MKDT median knock down time

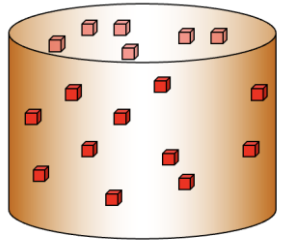
INTELLIGENT INSECT CONTROL

Applications for methods to determine surface concentration

- Product development – fast way to measure wash interval once minimum surface concentration is established with test systems
- Predicting efficacy without the constraints of mosquito strain differences
- Quality assessment of surface concentration post shipment
- Durability monitoring – check that surface concentrations still biologically relevant after 1,2,3 years

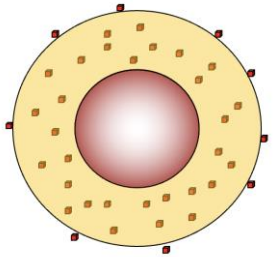
Two main ITN production methods

Incorporation:
insecticide mixed with a polymer to extrude mono filament yarn



Advantages: strength, durability
Producer controls yarn quality, knitting and incorporation
Limitations: not all insecticides can be incorporated

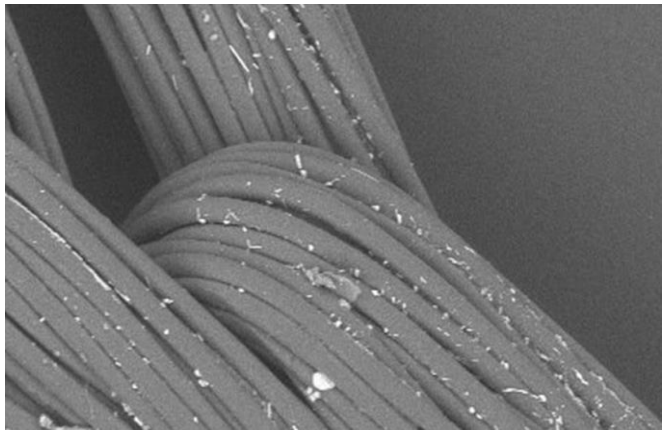
Surface treatment:
insecticide (mixed in a resin or a polymer) bound to netting fibres by dipping (or spraying) techniques



Advantages: ease of application, lower capacity costs
Nets mostly not in control of final producer,
Limitations: not all insecticides can be coated, difficult to add 2 AIs, multifilament yarns more fragile

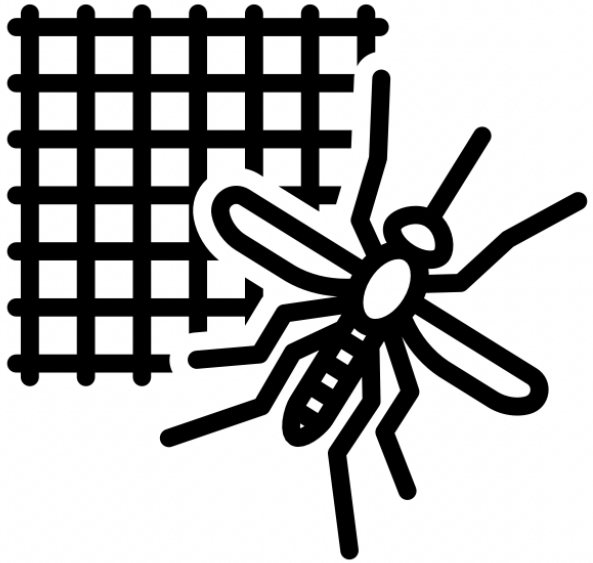
INTELLIGENT INSECT CONTROL

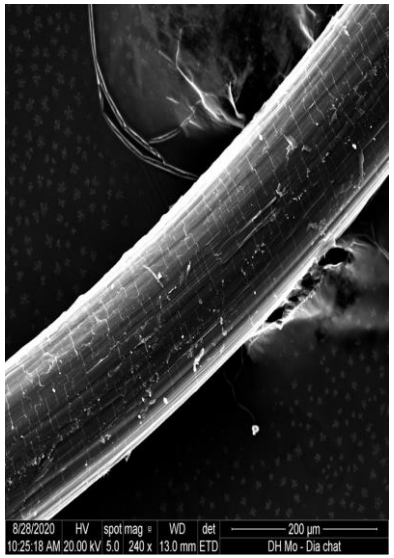
Evidently only insecticide at the ITN surface is relevant



Polyester
mulifilament yarn

Surface exposed insecticide



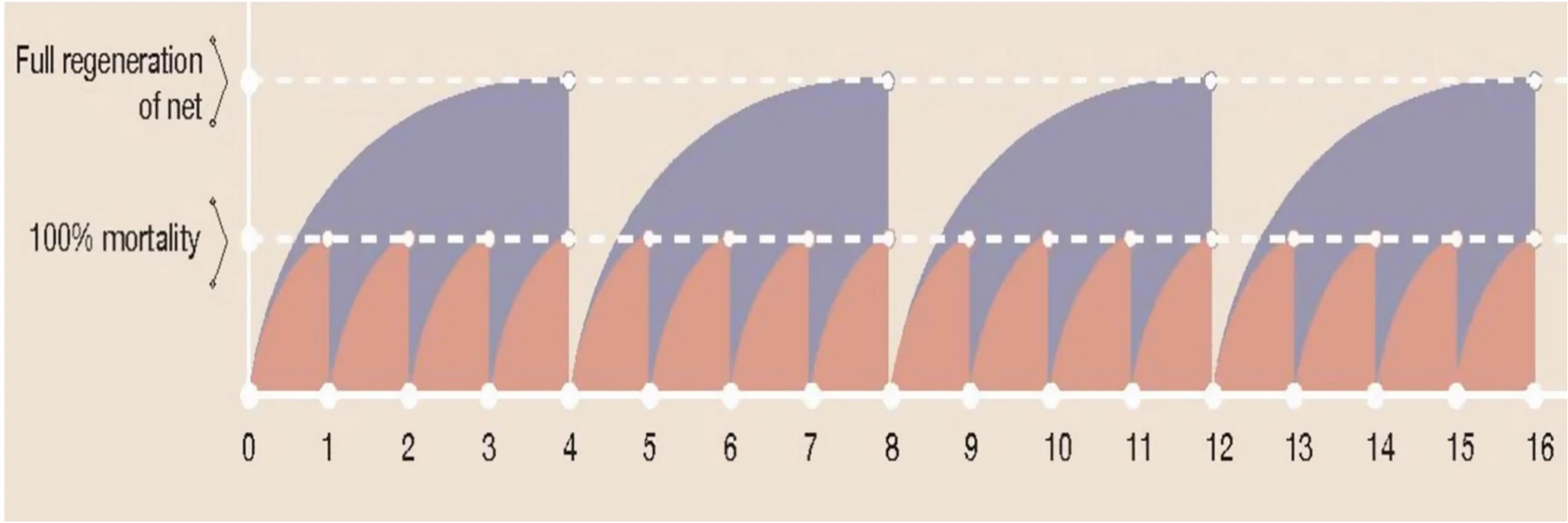


Polyethylene
Monofiber yarn

INTELLIGENT INSECT CONTROL

We need accurate means to measure surface concentration

- To correctly estimate regeneration time
  - “Data requirements and methodology for the Wash Resistance Index should be reviewed to consider the impact of selected wash intervals.”



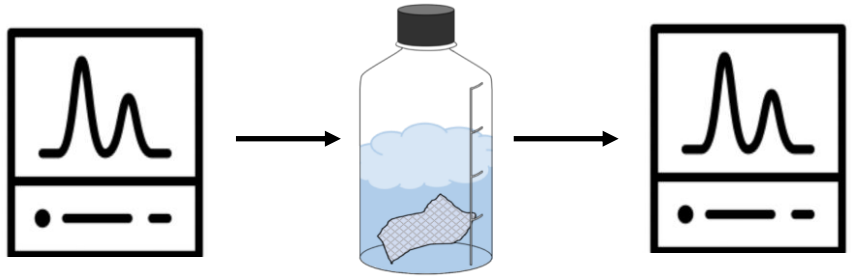
Skovmand *et al.* From the factory to the field: *Malar J* **20,** 363 (2021).

Two methods for determining surface concentration

BAM:

Before and After Method

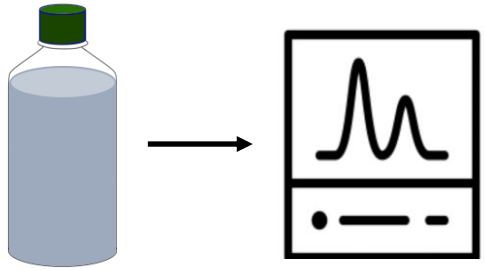
Measure total concentration before and after wash, the difference is what was washed off



SWAM:

Surface Wash Analytical Method

Analyse the surface wash off



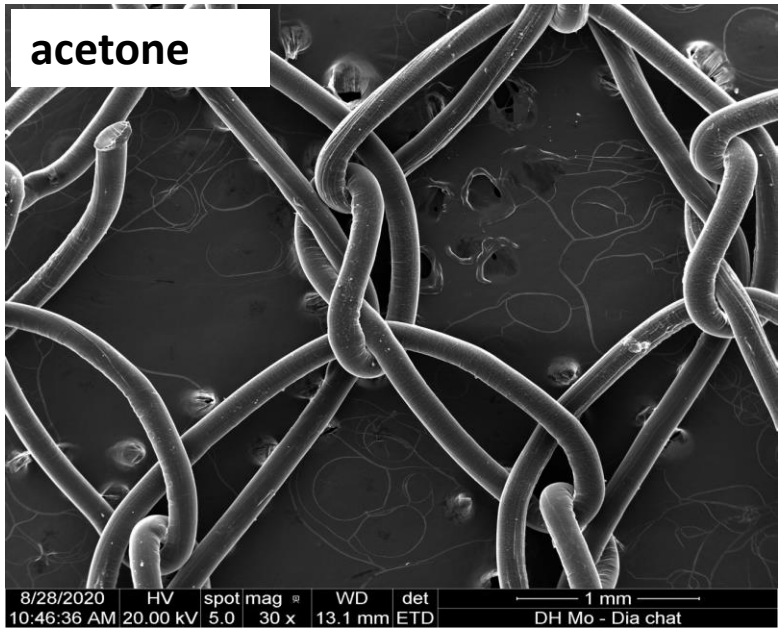
PE nets can be washed with cold acetone or soap water, coated nets may lose coating and surface insecticide

Nets with Deltamethrin or Alphacypermethrin form crystals at the surface

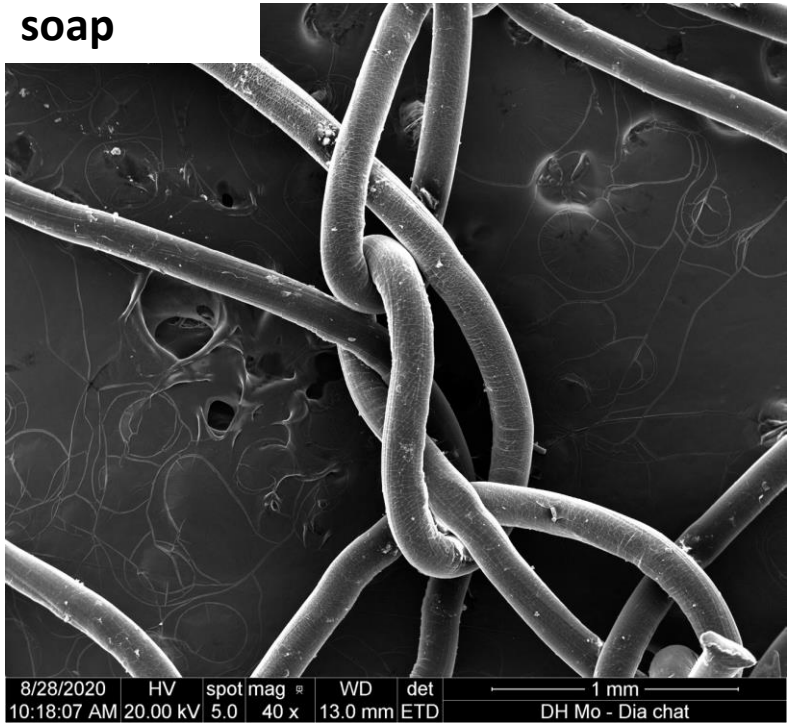
Acetone will remove all at the surface but not from inside the yarn.

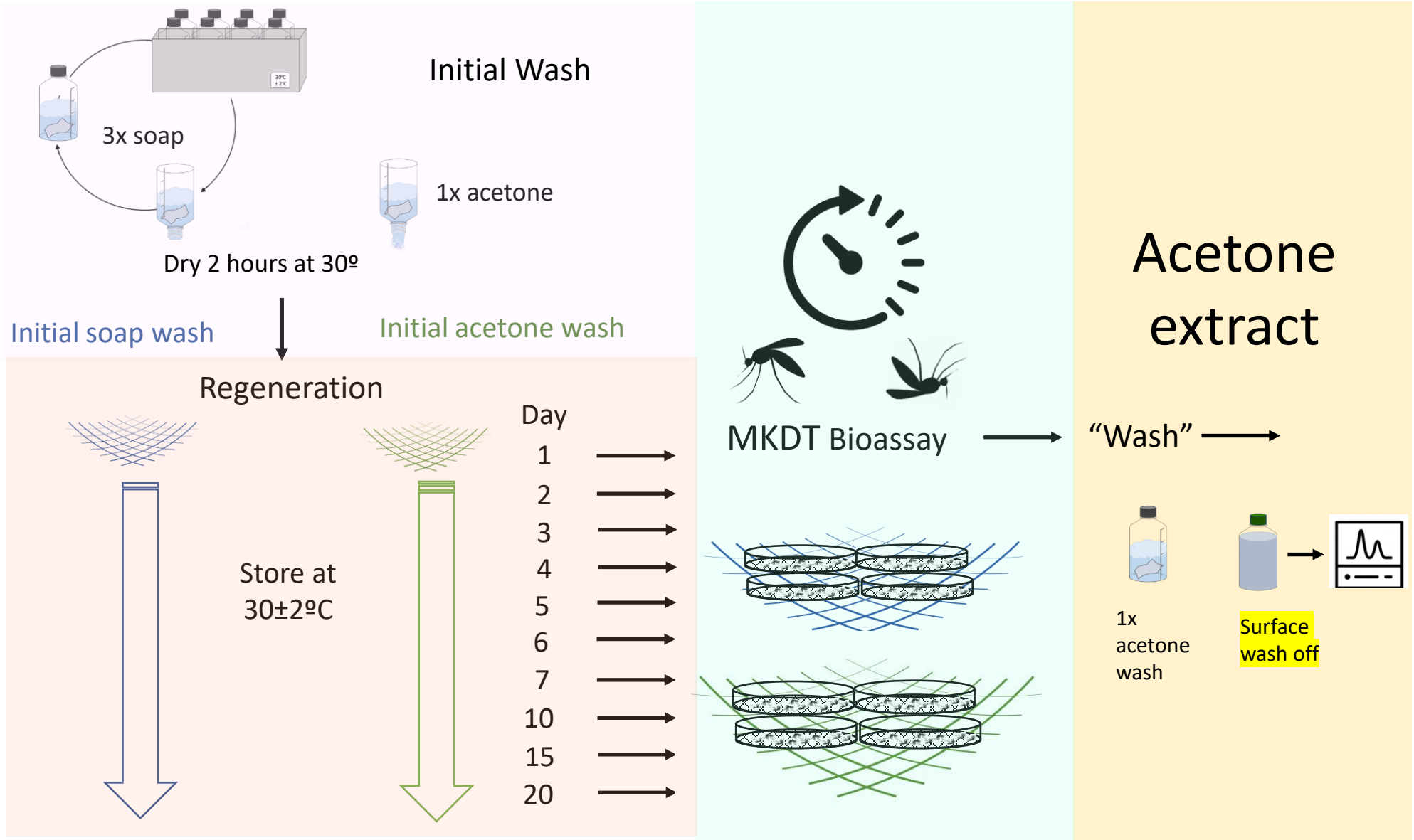
Soap wash will not remove all crystals, but removes all molecular (bioactive) insecticide

acetone



soap



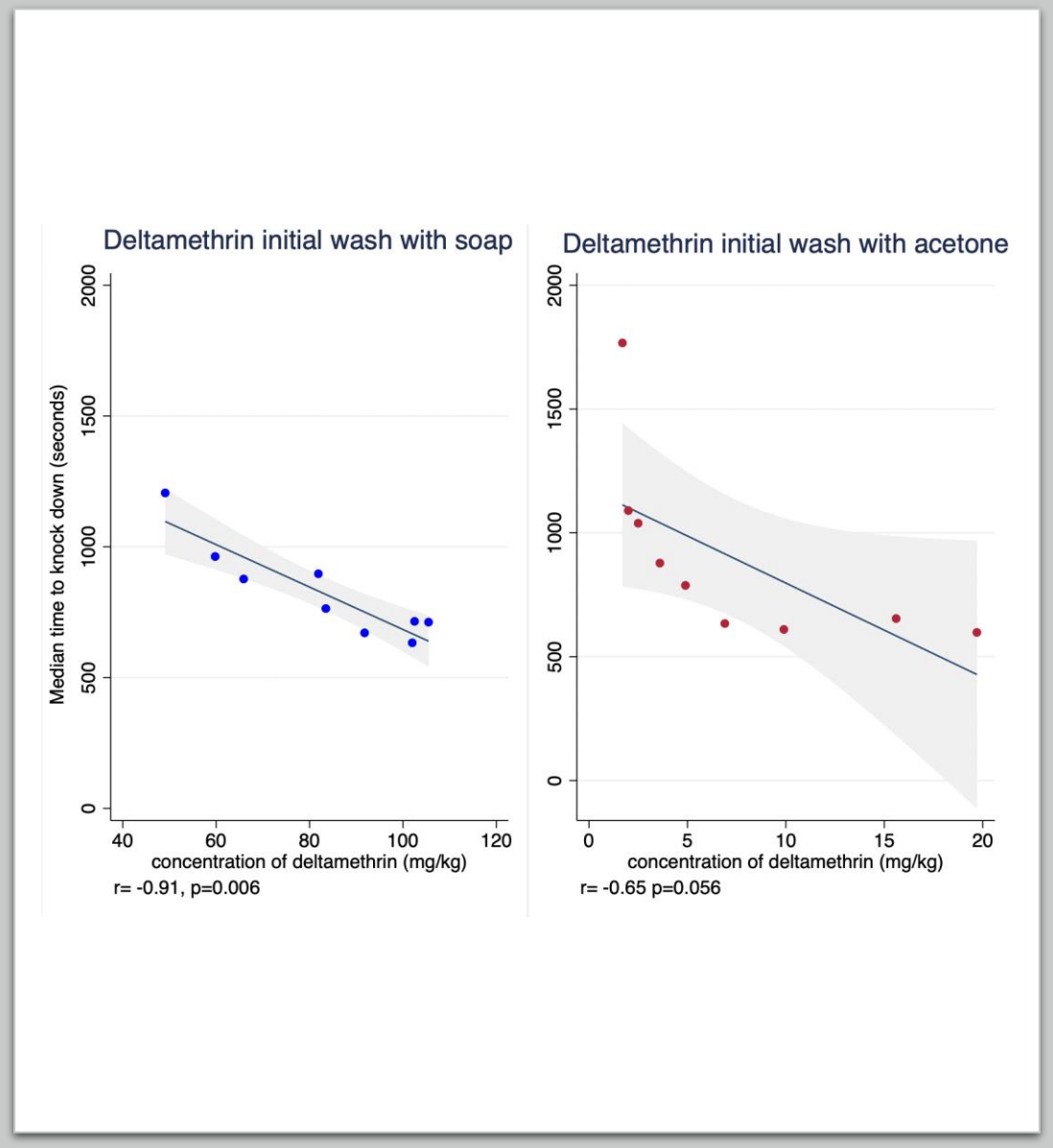


Surface Wash Analytical Method

Total surface content is determined from acetone extracts

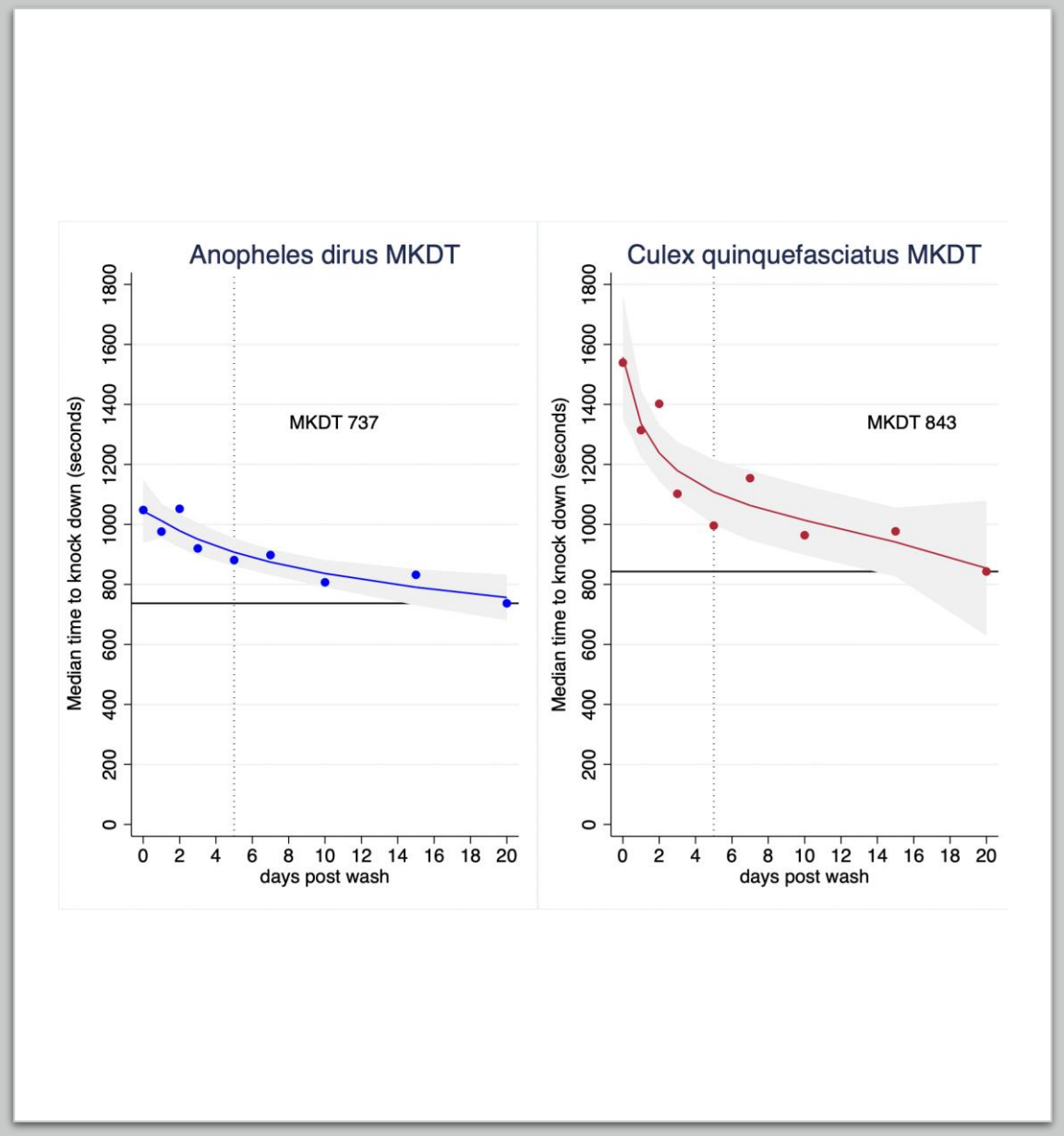
	After soap Wash			After Acetone dipping		
Days	PBO (mg/kg)	DM (mg/kg)	MKDT(Sec)	PBO (mg/kg)	DM (mg/kg)	MKDT(Sec)
2/24	25,4	49,1	1206	15,8	1,7	1767
1	70,1	65,9	877	36,2	2,0	1090
2	92,1	59,8	963	47,7	2,5	1039
3	119,5	81,9	897	59,6	3,6	878
5	134,0	83,5	764	71,3	4,9	788
7	119,5	91,8	671	68,7	6,9	634
10	136,6	105,5	712	84,6	9,9	610
15	175,1	102,5	715	115,8	15,6	654
20	185,4	102,0	633	109,9	19,7	598

Correlation between Median Knock Down Time and deltamethrin measured by SWAM was better with the initial soap wash

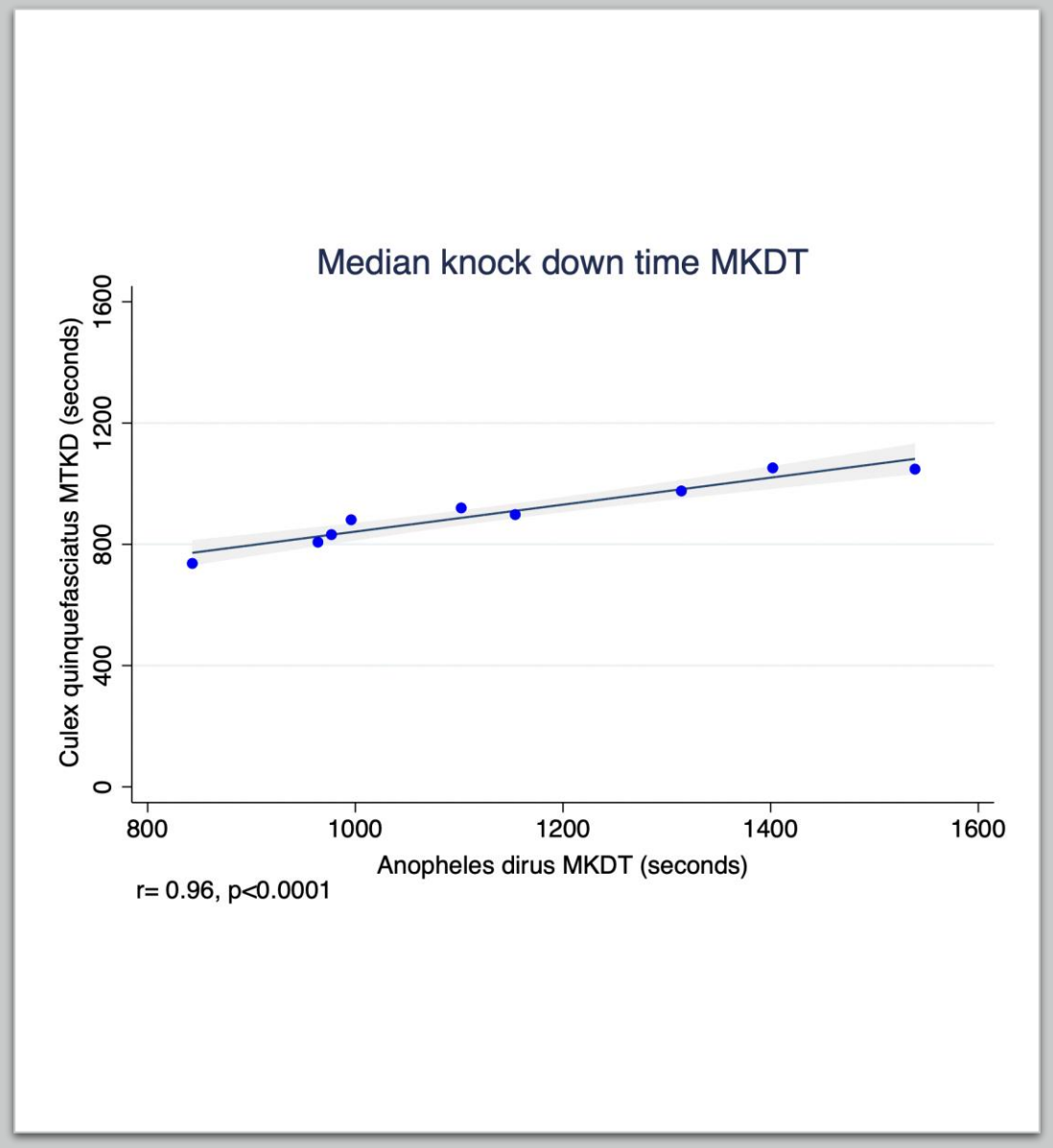


- When species with different sensitivity to PYR are used to test regeneration time the MKDT values are different but the number of days to a stable level is the same

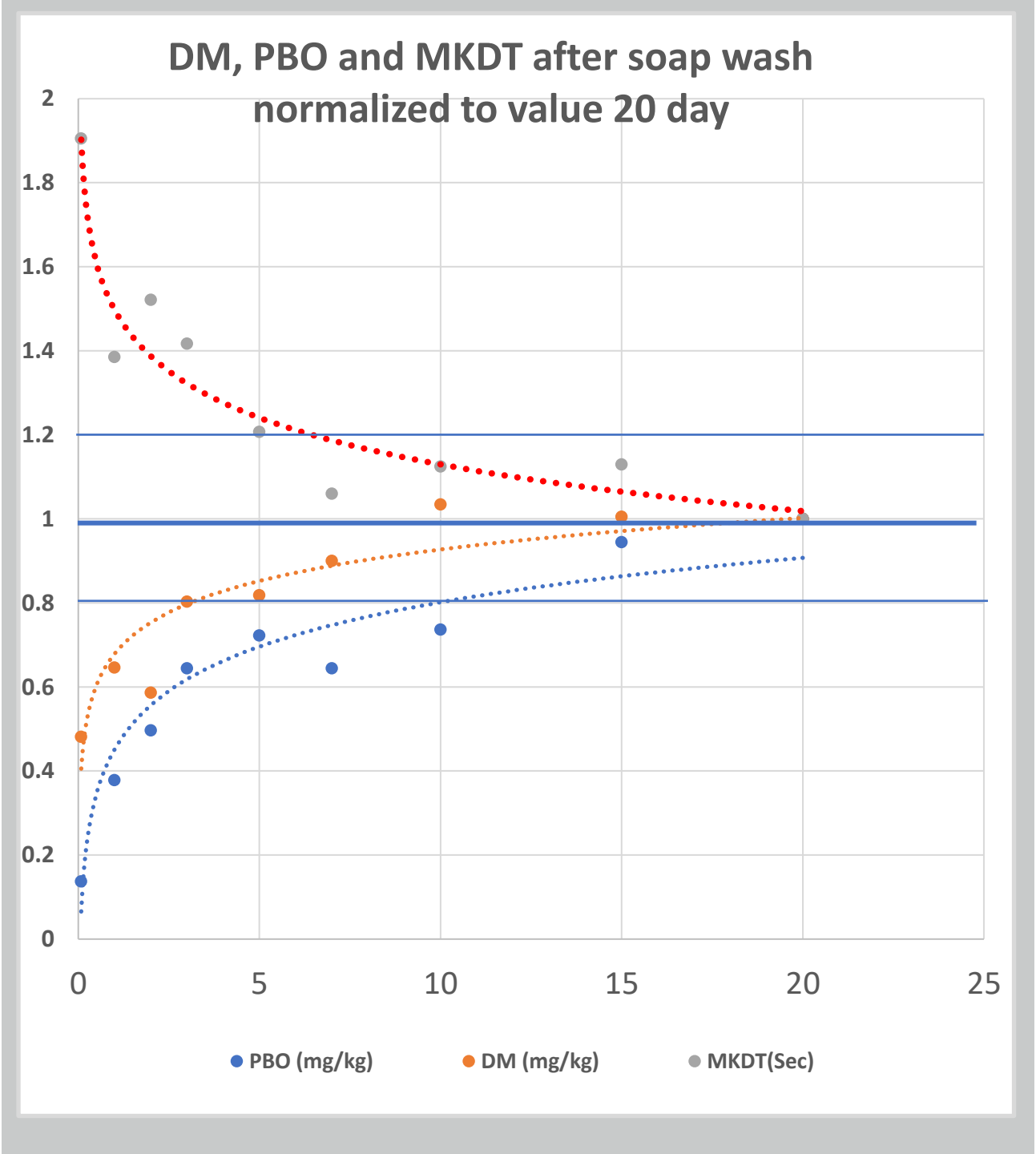
- In a classic WHO regeneration time, we would have 1 day for *An dirus* and 5 to 15 days for Culex



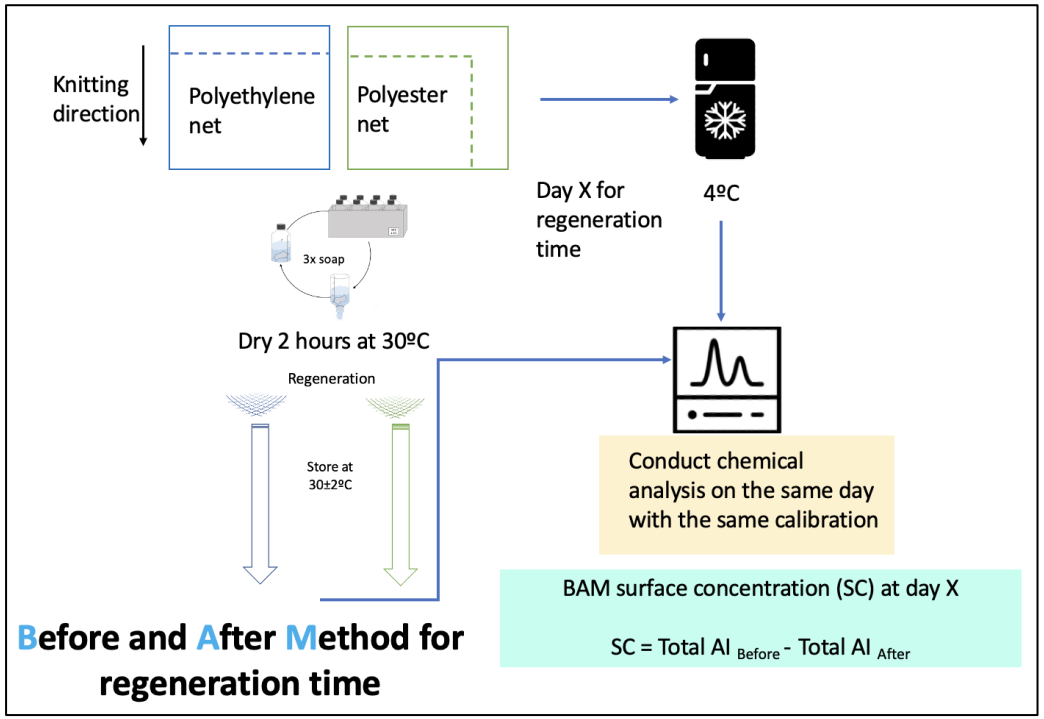
There was very high correlation between Median Knock Down Time (MKDT) for the susceptible and resistant strain

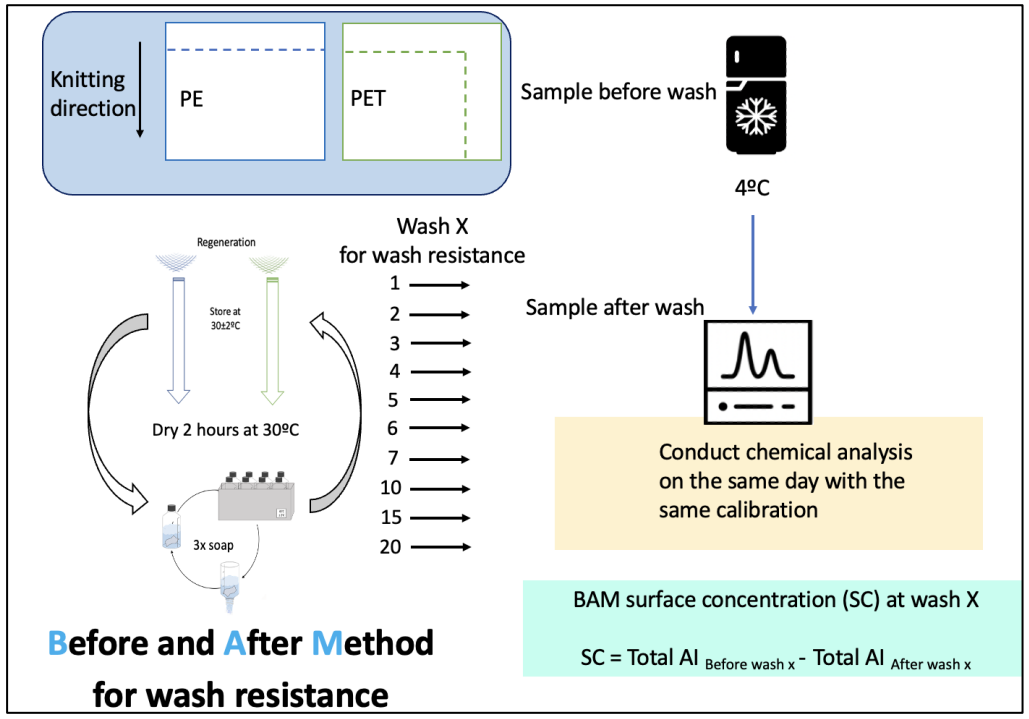


- The regeneration time measured by MKDT and regeneration of PBO and DM to the surface measured by acetone extraction
- Within 20% of the 20-day value by day 5 and fully regenerated by day 10



Before and after method



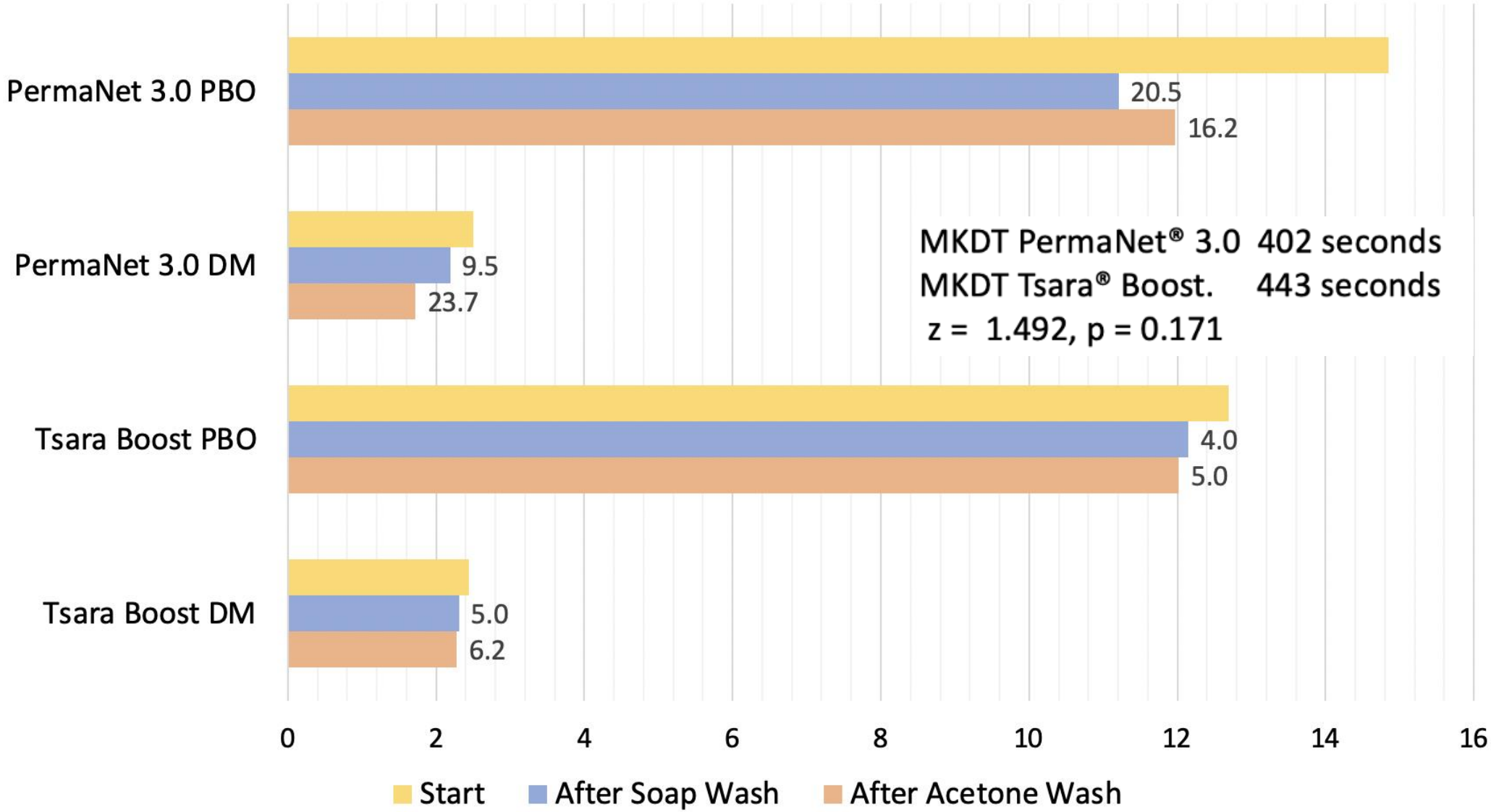


Classic WHO chemical analysis are BAM data and can be used to calculate surface concentrations

Evaluation of BAM

- Two commercial nets tested
- Wash off was conducted with either a CIPAC soap wash or dipping in acetone using SWAM
- Results were informed by bioefficacy testing with Median Knock Down Time (MKDT)

Surface concentration measured by before and after method (BAM). Wash off from soap or acetone wash measured in commercial ITNs. Data are grams per kilogram. Labels show surface concentration "out of the bag" as a percentage of total active ingredient



Comparison SWAM and BAM on 4 net samples, 2-4 determinations per sample

	Pbo		Deltamethrin	
Sample				
	Aver	std dev	Aver	std dev
1st wash water	459.38 (88%)	71.17	27.58(66%)	3.78
2nd wash water	30.82	1.93	7.66	0.96
3rd wash water	17.54	2.54	6.85	0.53
re-extract 1.st water	12.49	1.53	0.69	0.39
Nets before wash	13334.50	367.14	3608.83	252.97
Nets after wash	12603.25	404.76	3362.83	325.95
SWAM (sum of wash waters)	520.23	95.01	42.78	0.92
BAM (Total Before-After)	731.25	665.46	246.00	578.21

One BAM DM was negative

Two methods for determining surface concentration by washing

BAM

- depends on net homogeneity
- sample destructive
- can be used X time after a washing provided no evaporation

SWAM

- does not depend on net homogeneity
- not sample destructive
- access to soap water solution needed

INTELLIGENT INSECT CONTROL

Conclusions

BAM and SWAM can measure bio-relevant surface concentration of AI in ITNs

MKDT is a better method than cone test for determining regeneration time (RT)

Surface concentrations and MTKD correlates and provide the same RT, independent of resistance level of the mosquito strain used

Wash intervals were longer than measured by standard WHO method

Combining MTKD and SWAM or BAM could improve predictions of ITN performance and further validations are warranted

Use of a portable field-adapted liquid chromatographic system (c-vue machine) to determine deltamethrin surface level on insecticide-treated nets as part of a three-year durability monitoring in Mali

USE OF A PORTABLE FIELD-ADAPTED LIQUID CHROMATOGRAPHIC SYSTEM (C-VUE MACHINE) TO DETERMINE DELTAMETHRIN SURFACE LEVEL ON INSECTICIDE-TREATED NETS AS PART OF A THREE-YEAR DURABILITY MONITORING IN MALI

Prof Moussa BM CISSE

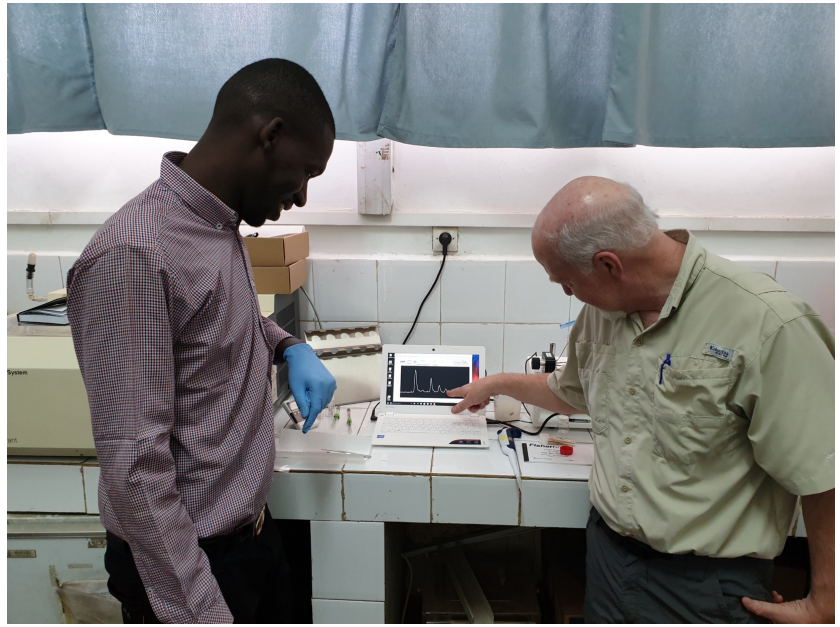
March, 19 th 2022

BACKGROUND

- The US President Malaria Initiative supported ITN durability monitoring in Mali from 2018 to 2020.

- The ability to monitor insecticide levels on ITNs is hampered in certain situations by the cost and availability of the equipment and resources required for analysis.

- LBMA in collaboration with CDC used a portable field-adapted liquid chromatographic system (C-Vue machine) to determine the surface level of deltamethrin on ITNs. Moreover WHO cone bioassays were used to determine ITNs bio efficacy.



OBJECTIVES

PRIMARY OBJECTIVE

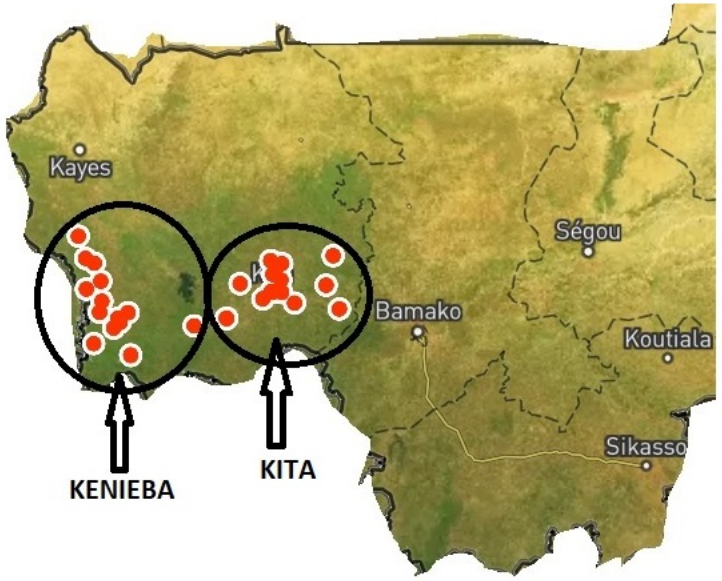
- Assess the insecticidal effectiveness of ITNs (Yorkool and PermaNet 2.0) in two villages at 12, 24 and 36-months after a mass net distribution

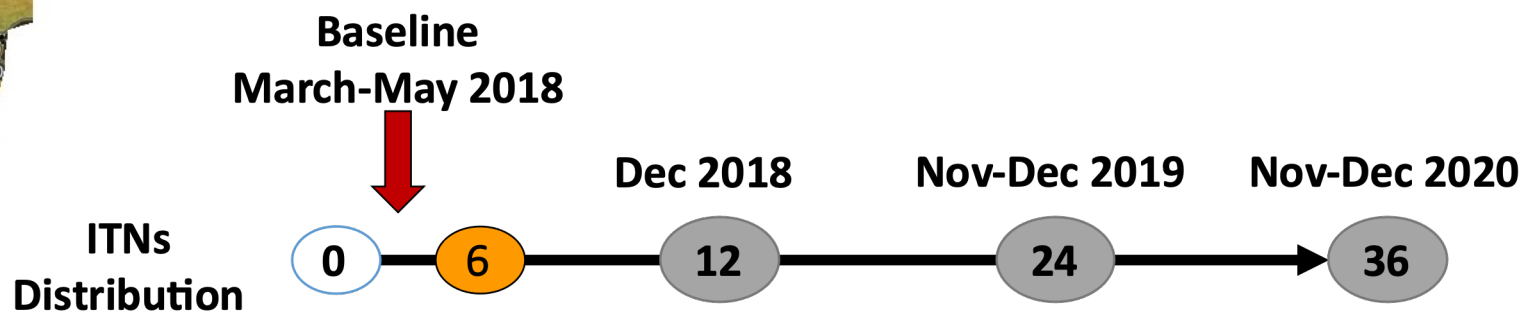
SECONDARY OBJECTIVES

1. Determine the nets handling, using and washing practices of the communities,
2. Assess the nets bio efficacy at 12, 24 and 36-months after distribution using WHO-Cone bioassays,
3. Estimate insecticide content at 12, 24 and 36-months after distribution using C-Vue HPLC method

METHODS 1/5

FIGURE 1. NETS COLLECTION SITES AND PERIOD





Subsample (30/ site) for bioassay and HPLC

Site	Net brand
Kenieba	Yorkool
Kita	PermaNet 2.0

METHODS 2/5

DETERMINATION OF NETS HANDLING, USING AND WASHING PRACTICES

The information about nets handling, using and washing practices was collected by using questionaries

METHODS 3/5

ASSESSMENT WITH WHO-CONE BIOASSAY

Laboratory-reared susceptible colony of *Anopheles coluzzii* (Ngousso)

Optimal effectiveness Knock Down 60 min ≥ 95% or **mortality ≥ 80%**

Minimal effectiveness : Knock Down 60 min ≥ 75% or **mortality ≥ 50%**

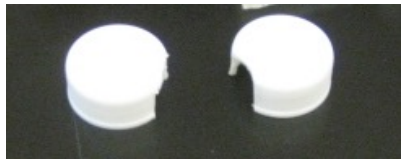
WHOPES criteria: at least 80% of recommended ITN brand should achieve optimal effectiveness 36 months post distribution

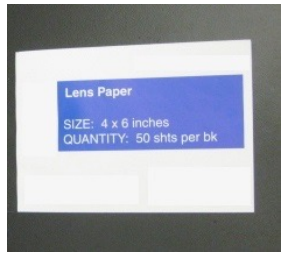
METHODS 4/5

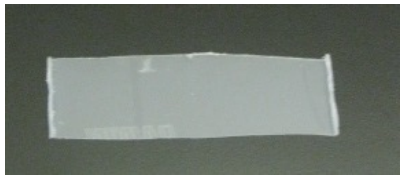
ASSESSMENT WITH C-VUE HPLC

SURFACE INSECTICIDE COLLECTION ITEMS

1. Spring-type wooden clothespins.
2. Sampling Caps (20-ml vial caps, 23 mm diameter, notched to accommodate clothespin).
3. Lens paper (100 x 50 mm for sampling and 25 x 20 mm squares for internal standard and calibration samples).
4. Bottle Cap (26 mm inner diameter, e.g. Coca-cola bottle cap).
5. Rubbing platform – 125 x 30 mm polypropylene plastic cut from the tops of 200-ul pipet tip container.
6. Binder clips – spring type, medium size with gripping lips made from plastic tubing.



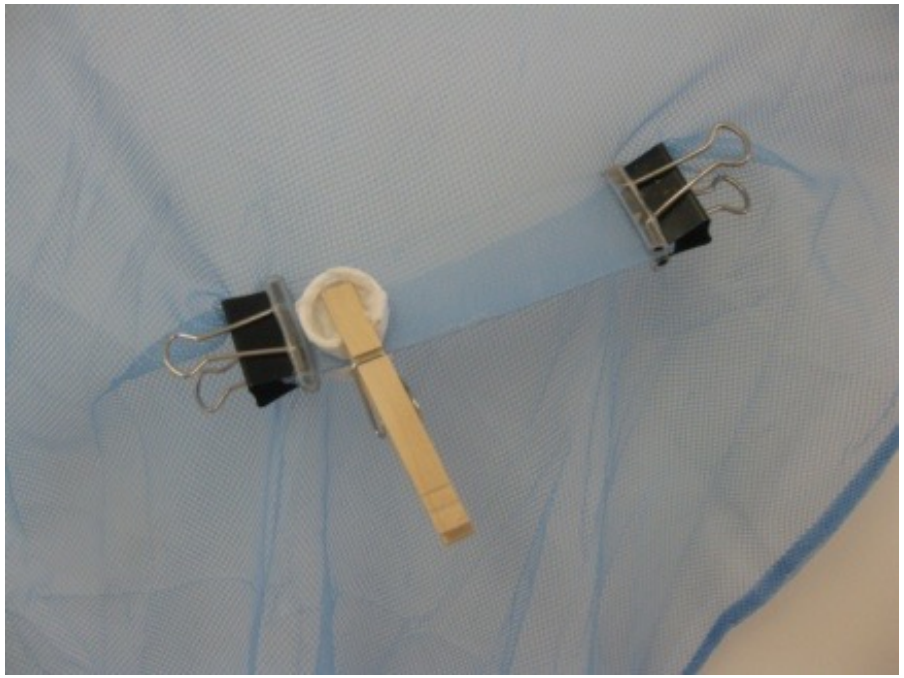




METHODS 4/5

ASSESSMENT WITH C-VUE HPLC

NET SAMPLING DEVICE USED TO COLLECT SURFACE LEVELS OF DELTAMETHRIN

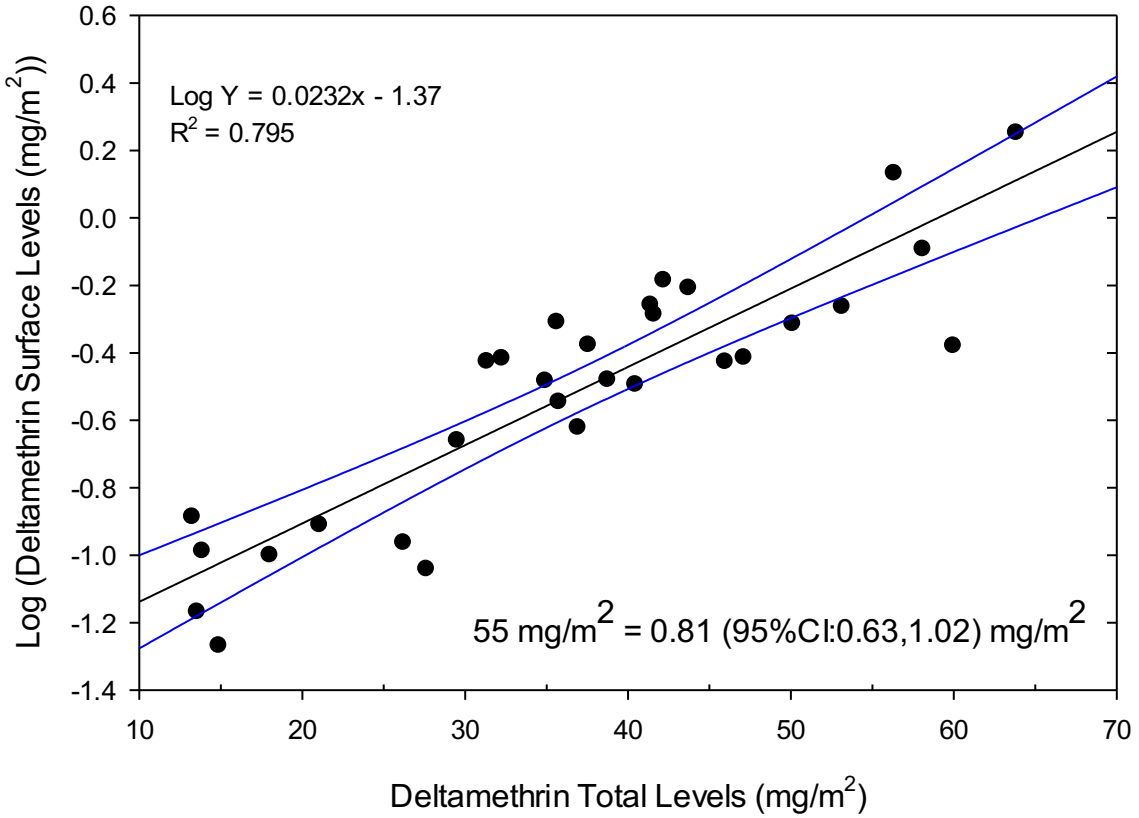


C-VUE HPLC COMPONENTS USED TO MEASURE DELTAMETHRIN SURFACE LEVEL

METHODS 5/5

ASSESSMENT WITH C-VUE HPLC

- The equation derived from the plot, Log Y = 0.0232x – 1.37, was used to estimate total deltamethrin levels and failure rate.
- The deltamethrin surface level associated with a new net containing the total level of 55 mg/m^2 is 0.81 mg/m^2 (95%CI:0.63, 1.02).
- A comparison for validation was made with the “Gold Standard” WHO-recommended HPLC technique for deltamethrin analysis (CIPAC)



RESULTS AND DISCUSSION 1/5

NET WASHING PRACTICES

TABLE. VARIABLES RELATED TO WASHING OF BIO-ASSAY AND CHEMICAL ANALYSIS TEST NETS

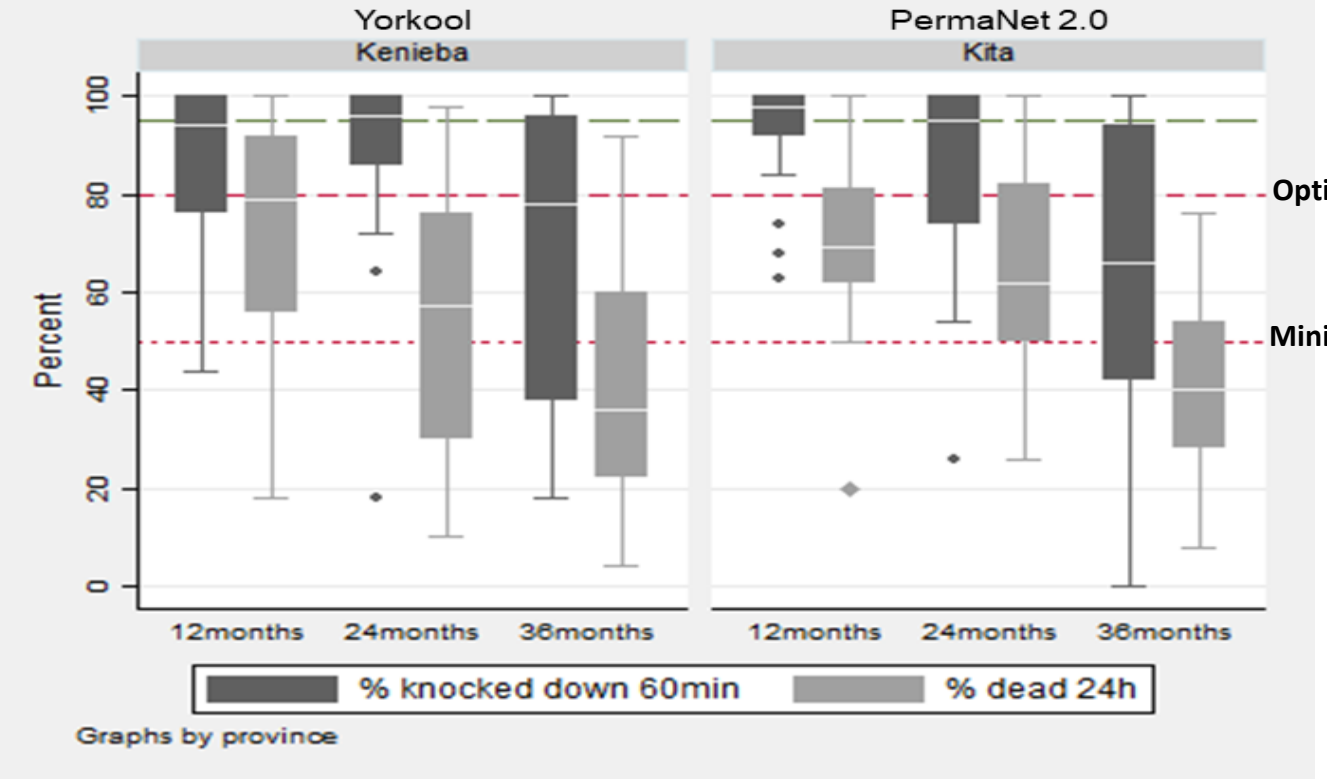
Variable	12 months	24 months	36 months
KENIEBA/ YORKOOL	N=30	N=30	N=29
Washes last 6 months (all)			
Mean	**3.2**	**2.8**	**2.0**
Median	1	2	2
Soap used			
country soap bar	38.1%	32.1%	21.7%
detergent or bleach	**52.3%**	**57.1%**	**43.4%**
mix	9.5%	10.7%	26.0%
Kita/ PermaNet 2.0	N=30	N=30	N=30
Washes last 6 months (all)			
Mean	2.4	3.1	3.0
Median	2	2	3
Soap used			
country soap bar	36.0%	37.9%	25.0%
detergent or bleach	**64.0%**	27.5%	**50.0%**
mix	0.0%	34.4%	3.5%

- **Nets had been washed an average of three times over the previous 6 months**
- **The main product used to wash them was detergent, bleach or regular bar soap in both sites**

RESULTS AND DISCUSSION 2/5

INSECTICIDAL EFFECTIVENESS USING WHO-CONE TEST

FIGURE. BOX PLOT OF CONE BIOASSAY USING A SUSCEPTIBLE COLONY OF *AN. COLUZZII* NGOUSSO ON YORKOOL AND PERMANET 2.0 ITNS



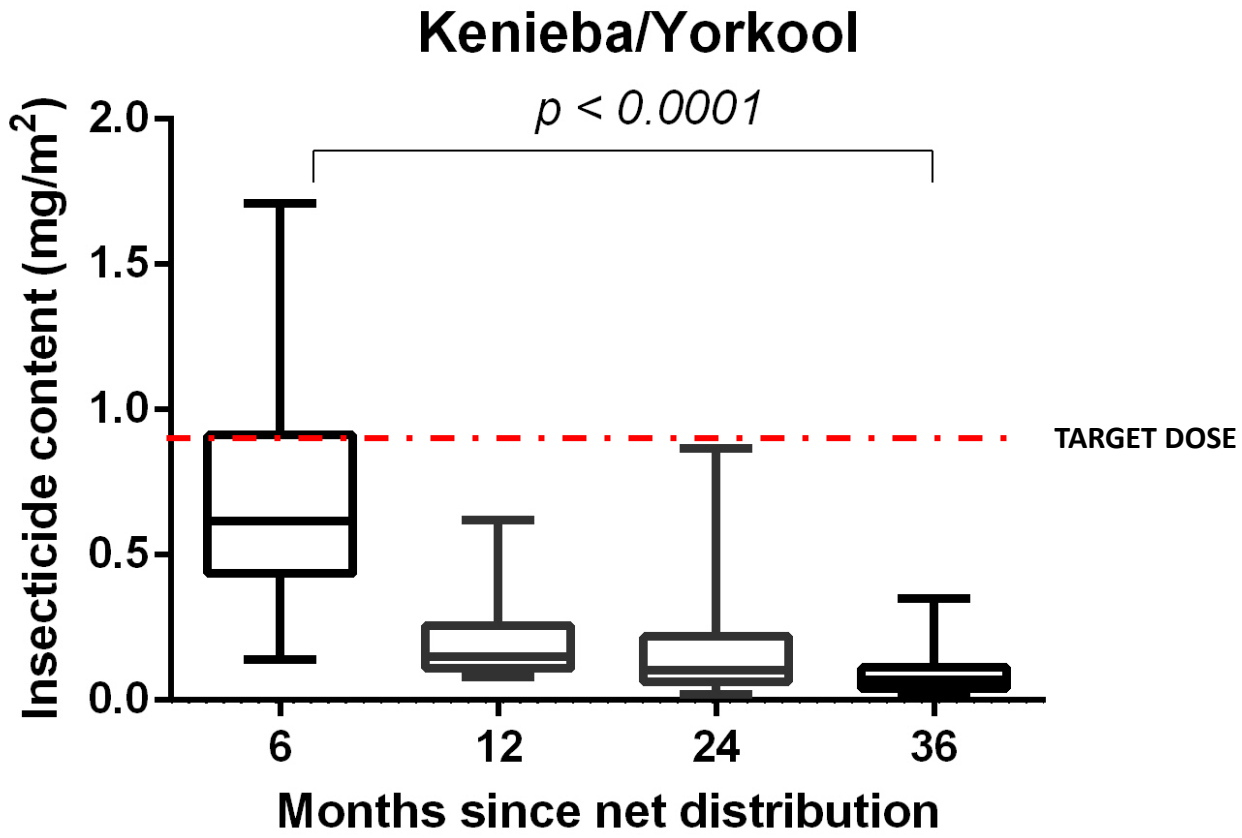
- **Optimal effectiveness (KD60 ≥ 95% or mortality ≥ 80%): < 80% at 12 –24 and 36-month for both brands. Based on WHO criteria there is a potential problem with low bio efficacy of both ITN brands**

- Minimal effectiveness (KD60 ≥ 75% or mortality ≥ 50%): >80% at 12 and 24-month for both brands

RESULTS AND DISCUSSION 3/5

INSECTICIDAL EFFECTIVENESS USING C-VUE HPLC

FIGURE. BOX PLOT OF ITNS CHEMICAL SURFACE LEVEL CONCENTRATION RESULTS USING A PORTABLE HPLC C-VUE



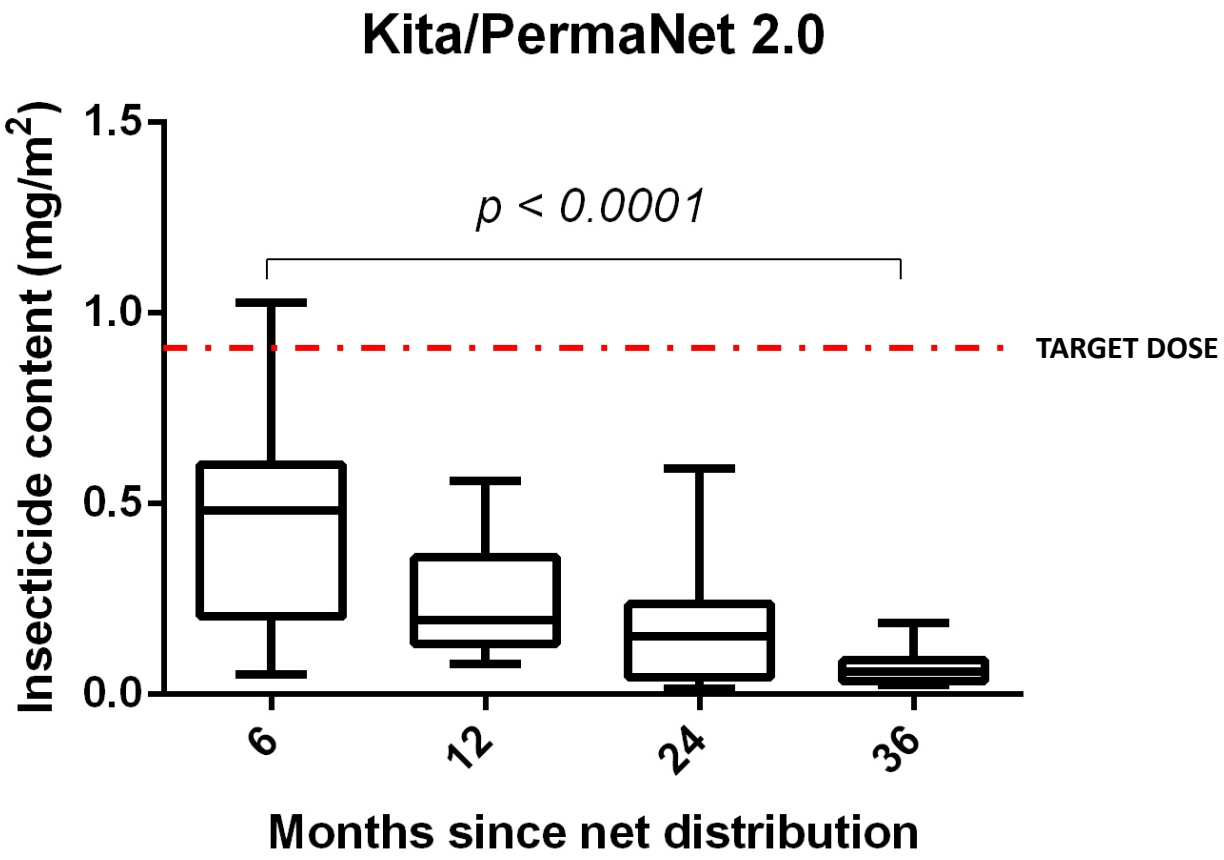
Deltamethrin surface level concentration decreased significantly (*p<0.05*) over the study period in both net brands. It has decreased from:

- **0.69 mg/m² at 6-month to 0.08 mg/m² at 36-month with Yorkool.**

RESULTS AND DISCUSSION 4/5

INSECTICIDAL EFFECTIVENESS USING C-VUE HPLC

FIGURE. BOX PLOT OF ITNS CHEMICAL SURFACE LEVEL CONCENTRATION RESULTS USING A PORTABLE HPLC C-VUE



Deltamethrin surface level concentration decreased significantly (*p<0.05*) over the study period in both net brands. It has decreased from:

- **0.46 mg/m² at 6-month to 0.06 mg/m² at 36-month with PermaNet 2.0.**

RESULTS AND DISCUSSION 5/5

INSECTICIDAL EFFECTIVENESS USING C-VUE HPLC AND CIPAC (GOLD STANDARD) METHOD

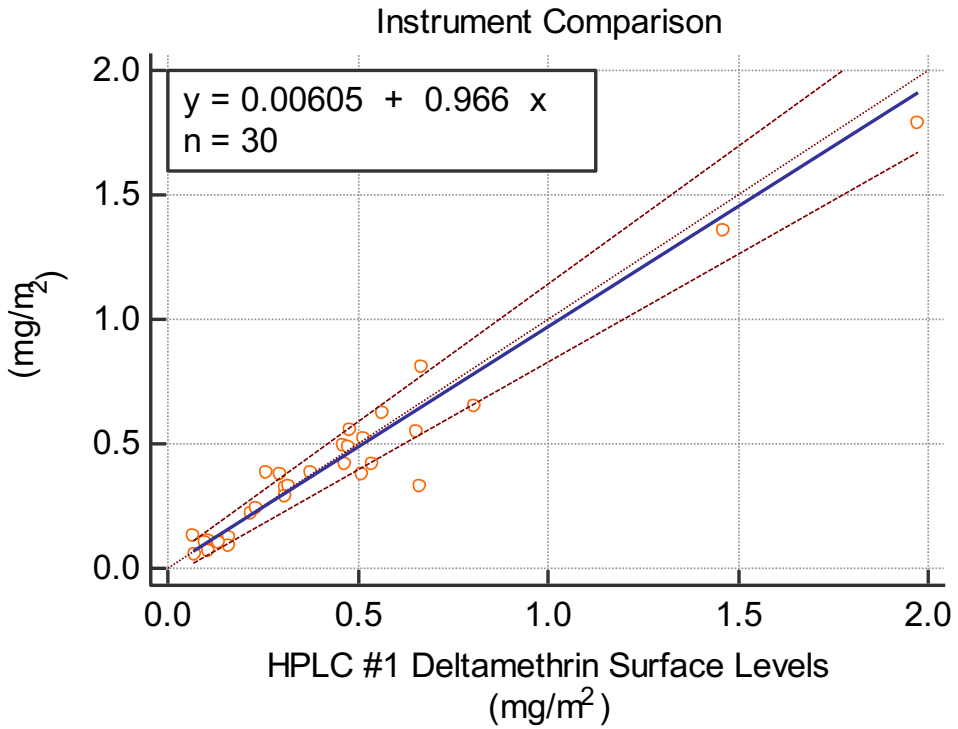


Figure. Passing and Bablok regression analysis for two instruments: instrument #1 is the Agilent 1200 Series, and instrument # 2 is the C-Vue® Portable Liquid Chromatograph Model CH2B.

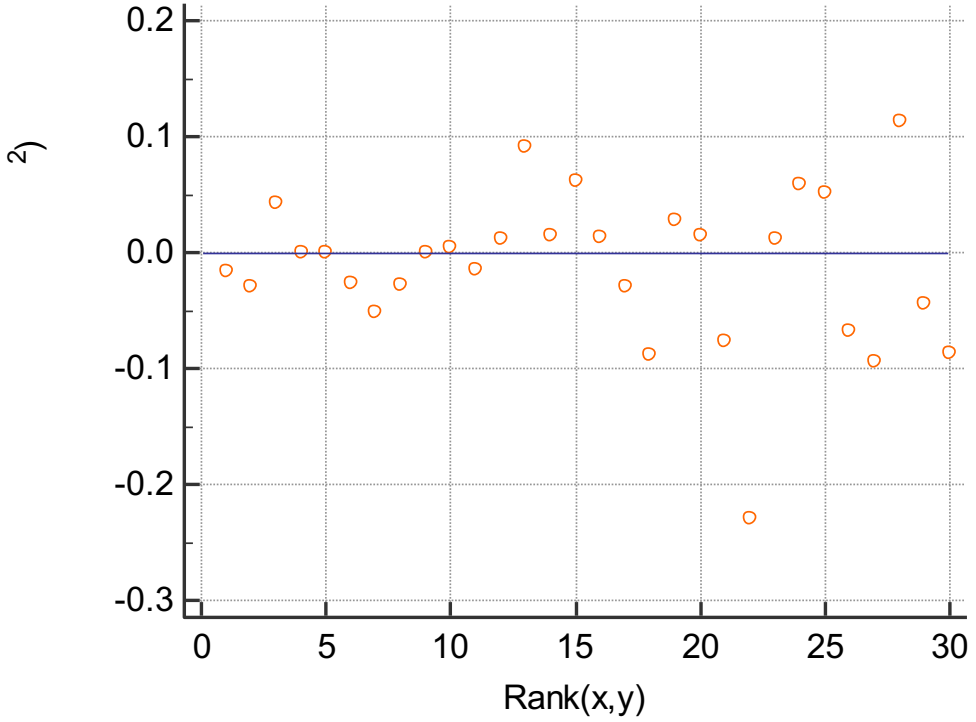


Figure. Residual plot of the distribution of differences around the fitted regression line.

CONCLUSION

- The use of detergent, bleach or regular bar soap in order to wash the nets were associated to the reduction of nets bio efficacy.
- The WHO standardized cone bioassay and C-Vue evaluation respectively demonstrated that the effectiveness and insecticide content of both net types were consistently lower than expected at 3 years.
- The C-Vue was used successfully for the first time in Mali to measure the insecticide surface levels of ITNs. Results that were consistent with cone bioassays and and gold standard HLPC methodology.
- This affordable new technology is less cumbersome than the standard HPLC typically used to perform to ITNs chemical analysis for durability monitoring in malaria endemic countries.

Perspectives

- Propose the C-vue method for ITN durability monitoring in malaria endemic countries
- Validate the use of C-vue method determine the surface level of PBO or chlorfenapyr, pyriproxyfen on ITNs

ACKNOWLEDGMENTS

PMI | U.S. PRESIDENT'S MALARIA INITIATIVE

LED BY USAID FROM THE AMERICAN PEOPLE

CDC

LBMA
LABORATOIRE DE BIOLOGIE MOLÉCULAIRE APPLIQUÉE

U.S.T.T-B

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

PROGRAMME NATIONAL DE LUTTE CONTRE LE PALUDISME
PNLP

THANK YOU

Overview of charrette group inputs

Challenges & Solutions -- Arrival of ITNs in country

	Challenges	Solutions	Implementation ideas
Pre-shipment inspection	• Trust deficit in the pre-shipment testing process	• Rotation of inspectors to be built into the system • Transparent and open data sharing of pre-shipment assessments	• Procurers to stipulate rotation/ best practice in contracts with 3rd party inspectors • Look at ways to share data more widely, such as procurers sharing data between themselves and with programs
Post shipment inspection	• Non-standardized post-shipment testing may cause rejection of good products or acceptance of poor products	• Develop harmonized guidance on pre- and post delivery inspection criteria and SOPs	• LQAG (procurers QA group), WHO PQ and NMCP partners working group • Need to understand what inspection is done post shipment
QMS	• Balance and optimise roles of QMS and inspections to drive ITN quality improvements	• Build trust and understanding • Define intended purpose and rationale of QMS and inspections from multiple stakeholder perspectives • Full implementation of ISO 9001	• Produce advocacy brief on QMS and inspection • Clear definitions between testing, inspections and QMS to be developed and used by all partners

Challenges & Solutions -- Arrival of ITNs in country

	Challenges	Solutions	Implementation ideas
Port delays and storage	• Delays and storage at port including customs clearance and distribution related delays • Storage conditions may need to be tailored to individual products • Landlock-country challenges are magnified (e.g. tracking and container availability)	• Understand magnitude and impact of this challenge • Advocacy to facilitate rapid customs clearance • Definition of and guidance on optimal storage conditions (net specific where necessary) • Audit, upgrade and improve storage capacity • Improved demand forecasting to facilitate greater adherence to delivery date forecasts and storage planning	• AMP may have information/data on impact/scale of problem • Research collaboration between academia and GF/procurers/shipping companies • Potential use of contract to define conditions during shipping • NMCP/MoH to contact customs agencies • Manufacturers to provide information as part of PQ dossier • Donors and funders may need to support storage upgrades
In-country testing capacity	• Insufficient in-country capacity: funding, staff infrastructure and other resources for conducting ITN testing.	• Facilitate countries with an interest in conducting optional in-country quality tests • Ensuring countries have most up to date SOPs and other resources to support methods	• Secure funding for countries to develop the capacity to conduct independent tests – *to define the need for tests and responsible parties within this*

Challenges & Solutions -- Distribution of ITNs

	Challenges	Solutions	Implementation ideas
Usage/ Care	• Lack of proper care and use instructions • Lack of BCC/budget constraints for BCC	• Develop guidelines for care and use and include in relevant labelling • Develop appropriate BCC strategy/sensitization (pre-, during, post-distribution	• Community engagement based on feedback/listen to communities • Provide funding for BCC • More data on ITN usage using new tools and approaches to collect (e.g., accelerometer approach)
Storage & Transport	• Inappropriate storage/transport conditions at different levels (national and subnational) • No guidelines for warehousing • Budget constraints, including lack of domestic & private sector resources, impede proper storage/transport	• Invest in warehouse infrastructure • Research on impact of storage conditions on performance • Agree and enforce guidelines and SOPs surrounding storage and transport at national and sub-national level • Enforce quality assurance at different steps in the supply chain	• Develop guidelines for inspection of storage facilities and for response to non-conformity in transport/storage • Phased delivery to reduce storage time • Train NMCPS on net storage • Suppliers to lead post-market surveillance • Data generation to inform engineering of nets with better resistance to temperature and humidity

Challenges & Solutions -- Distribution of ITNs

Distribution Strategy

Challenges	Solutions	Implementation ideas
• Lack of clear distribution strategy/microplans and delays in distribution at different levels perhaps leading inappropriate storage • Oversupply of nets and associated use of old nets • Over-simplification of 3-year campaign cycle and sufficient data to inform adjustments	• Develop clear/proactive distribution strategy/ microplanning • Digital real-time data collection systems/proper data capture and use/Digital tracing of nets • Quality management of distribution channels • Flexibility of funding for countries to adjust campaign cycle based on durability data	• Engagement with AMP efforts around coordination of stakeholders for guidance and distribution planning/learning around best practice.

Challenges & Solutions -- ITN usage and data generation

	Challenges	Solutions	Implementation ideas
Available data	• Lack of data on performance/ bio efficacy • Insufficient data on durability monitoring/risk factors in country that influence life of a net • Lack of information on how performance of nets evolve over time and how this is linked to specifications	• Post market surveillance on retention, bioefficacy, AI concentration, physical integrity and use • Publication of data to make appropriate data available to all	• Funding for data collection • Need to define as a community what data is needed and what data is already available for post market surveillance
Data use	• Feedback loop not sufficient for informing decisions based on data • Data biased (perception or real) • Capacity to assess bioefficacy of new ITNs in country is limited (if post distribution surveillance should be implemented)	• Feedback system in place for durability monitoring -> PQT -> ITN manufacturing -> product improvement • Funding for entomology labs	• Data flow used to inform user-centered design updates to products [as a community to work with manufacturers to define data needs and use cases for product updates (fixing problems, innovation and incentives for innovation)] • Funding for analysis of existing data

Challenges & Solutions -- ITN usage and data generation

	Challenges	Solutions	Implementation ideas
Lack of common vocab	• Lack of clarity of what post market surveillance is for • Misinterpretation of specifications, what they represent and when they should be applied • Unclear distinction between quality and performance	• Develop glossary of terms • Communication of and commitment to glossary by stakeholders	
Usage by end user	• Less than optimal handling of nets (drying practices, frequency of washing, methods of washing, environmental stressors) • Risks to net during storage • Inadequate repair of nets	• Effective communication through the right channels on appropriate use and care • New ways of collecting relevant data to inform user-centered design for products • Funding for SBC	• Clarify value of increased lifetimes [work ongoing] and translate that to flexible processes that can be informed by data [donors, procurers, countries]
Data quality	• Concerns around quality control of data collection & design of surveys	• Collaboration around design protocols for surveys	

Challenges & Solutions -- ITN usage and data generation

	Challenges	Solutions	Implementation ideas
Guidance	• Lack of sufficient guidance on handling nets • Guidance is available but not used (e.g., packaging lost/not aligned) • Lack of guidance on performance standards for nets & associated lack of incentive to improve performance due to lack of standards	• SOPs/guidelines for use of bed nets • Specify what performance standards are needed and/or reasonable and how they would be used	• Technical solution: indicator for monitoring number of washes • Better instructions and labelling on packaging • Consensus building on reasonable expectations for performance standards
Context	• Food insecurity leading to mis-use of new nets • Insecurity, flooding & natural disasters leading to loss/destruction of nets • Housing construction • Rodents • Access to nets affecting retention time	• Promote/incentivize durable net design • Review how we can be flexible for procurement and distribution given established variability of net duration in the field •	

Theory of Change

- Generally resonated with people
- Suggested updates to focus and clarity in some areas
- Roles, responsibilities and timelines needed

User centered approach to surveillance

- Can inform ITN design, specifications and SBC activities
- What data do we need to get and what tools do we have to collect it?

QMS implementation support

- Governance of ISO 9000 module implementation
- Raw material specifications and inspections

Build out cross cutting activities

- Foundation on which the ToC rests and driver of trust
- Identify how key data can be shared and interpreted, particularly change management

Need to emphasize price

- Understand incentives to drive innovation
- What data/characteristics can drive value-based procurement?

Discussion: does this summary reflect your discussions? Is there anything missing?

Consolidated focus areas and next steps

Key focus areas for post-shipment ITN quality issues

Post-market surveillance

1. Definition of terms for post-shipment surveillance, what this includes, what data is available and what is needed
2. Building trust in pre-shipment inspection: Standardization of tests, Stipulation of best practices for third party inspectors, Data sharing for pre-shipment assessments, Advocacy brief on QMS and auditing implementation of ISO-9001

User-centered design

3. Data on user preferences and usages to inform product updates, including to fix identified problems and to support innovation.
4. Clarify value of increased lifetimes [work ongoing] and translate that to flexible processes that can be informed by data [donors, procurers, countries]
5. Review design factors, labelling and packaging to support appropriate handling for users

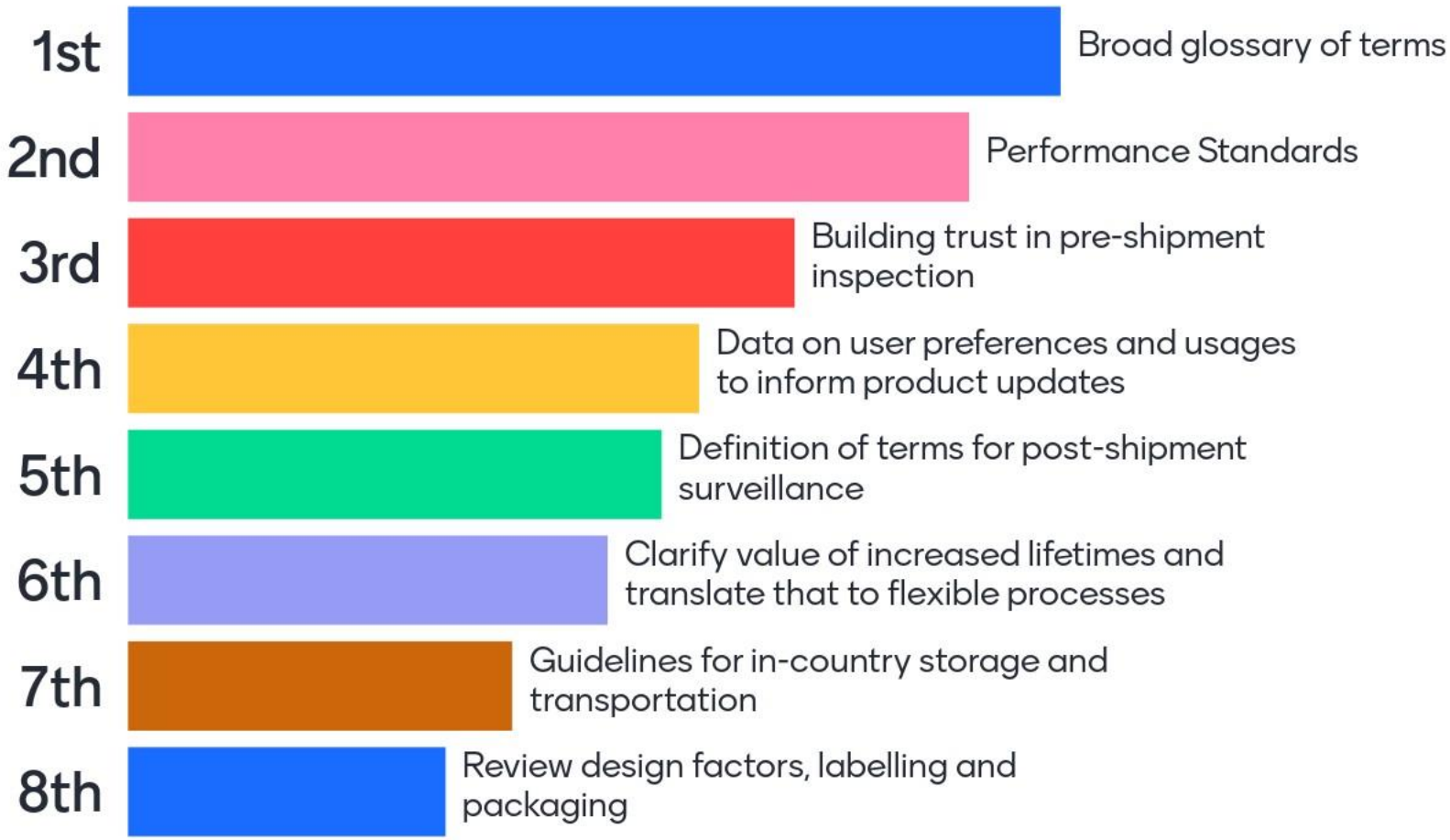
Cross-cutting issues

6. Broader glossary of terms covering pre and post shipment quality processes
7. Performance standards: what can reasonably be expected from performance standards and how can user data be incorporated?
8. Guidelines for in-country storage and transportation of nets, including for inspections of storage facilities and response for non-conformity

Discussion: any key focus areas/activities you think are missing here?

What priority would you assign to the following items?

Mentimeter



Next steps for post-shipment quality concerns

Themes	Activities	Next steps	Lead/Key stakeholders	Timelines
Cross-cutting	Develop a clear glossary of terms	PQ working on these		
Global policy guidelines - Minimum standards	Develop specifications that link net quality to performance	PQ working on these		
Procurement - tendering	Document and measure characteristics that lead to better performance and have this reflected in procurement decisions.	Eddie to update		
Convening 2	Glossary of terms	PQ working on this Communication plan and review process		
Convening 2	Performance Standards			
Convening 2	Building trust in PDI	Sharing data more broadly, need better understanding of where trust issue comes from and clarifying process		