#### Ivermectin

#### should we rethink its toxicity?

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#### Outline

- Avermectins vs Ivermectin (IVM)
- Usual dose as pesticide
- Types of toxicity
  - cytotoxic damages cell functions partially/fully
  - genotoxic damages nucleic acids partially/fully

R.W. Burg, B.M. Miller, E.E. Baker, J. Birnbaum, S.A. Currie, R. Hartman, Y.L. Kong, R.L. Monaghan, G. Olson, I. Putter, J.B. Tunac, H. Wallick, E.O. Stapley, R. Oiwa, S. Omura, Avermectins, new family of potent anthelmintic agents: producing organism and fermentation, Antimicrob. Agents Chemother. 15 (1979) 361– 367.

- Streptomyces avermectinius, produces the anthelmintic macrolide "avermectin" that was isolated by Omura et al. of the Kitasato Institute from the soil sample collected in Ito City, Shizuoka Pref.
- The Streptomycetes bacteria produce various antibiotics such as Streptomycin, Erythromycin, Tetracycline etc.
- These bacteria produce 60% of naturally-occurring antibiotics and are used as antibacterial, antifungal, antiviral, anitiparasitic, immunosuppressant and antitumor medicines.

Br. vet. J. (1980), 136, 88

#### Ivermectin

#### Synonyms:

MK-933, 22,23-Dihydroavermectin B1, Ivermectin

#### 22, 23–DIHYDROAVERMECTIN B<sub>1</sub>, A NEW BROAD-SPECTRUM ANTIPARASITIC AGENT



• Merck chemists made a minor chemical modification to Avermectin B1 to create ivermectin, which was marketed in 1981 for animal use.

## Soap vs Ivermectin

- Soap = 450 g coconut oil + 79g NaOH + 170 g water
- Can we assume that soap is safe to consume, because it is chemically derived from coconut oil ?
- Can we assume that Ivermectin (IVM) is safe because it is chemically derived from the fermented products of Streptomyces avermectinius ?

# IVM dose as pesticide

- 0.15 mg per kg body weight normally given once a year, or twice a year (rarely thrice a year).
  - For a person weighing about 60 kg, this is about 9 mg of IVM. (some makers provide 3 mg tablets, some provide 12 mg tablets)
  - Sources:

1.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4425412/

2. https://www.mayoclinic.org/drugs-supplements/ ivermectin-oral-route/proper-use/drg-20064397

## 'COVID-19' IVM protocol

- Zelenkov 0.4-0.5mg/kg/day for 5-7 days (<u>https://</u> <u>vladimirzelenkomd.com/treatment-protocol/</u>)
- Mc Cullough 0.2-0.6 mg/kg/day for 2-3 days (<u>https://</u> <u>rcm.imrpress.com/article/2020/2153-8174/RCM2020264.shtml</u>)
- For a person weighing 60 kg, this is about 30 mg/day taken for 5 consecutive days.
- This dose is not only 3 to 4 times strong compared to what humans take, but also given too often.
  - Is this safe for humans to consume?

### Safety of IVM in animals

- How do animals respond to strong doses of IVM?
- Animals tolerate toxicity differently from humans. Therefore, such results should always be cautiously interpreted.

#### Animals vs humans

- In general, animals tolerate toxins much better than humans.
  - this may be due to multiple reasons
    - for example, animals have superior ability to generate antioxidants such as ascorbate (vitamin c) or glutathione. Thus they are better protected against oxidative stresses compared to humans.
    - rate of metabolism (the ability to digest products that enter into the cells and tissues of living beings) is generally lower in humans.

#### Human to animal dose taking body surface area into account

Human equivalent dose calculation based on body surface area\*

#### Species To convert animal dose in mg/kg to HED Reference Working Body To convert dose in weight mg/kg to dose in in mg/kg, either body surface mg/m<sup>2</sup>, multiply by K<sub>m</sub> weight (kg) range (kg) area (m<sup>2</sup>) Divide animal dose by Multiply animal dose by Human 60 1.62 37 12.3 Mouse 0.02 0.011-0.034 0.007 3 0.081 0.08 0.047-0.157 0.016 5 7.4 0.135 Hamster 0.025 6 6.2 Rat 0.15 0.08 - 0.270.1625.3 Ferret 0.300.16-0.54 0.043 7 0.1898 4.6 0.40 0.208-0.700 0.05 0.216 Guinea pig 3.1 Rabbit 1.8 0.90-3.0 0.15 12 0.324 Dog 10 5-17 0.50 20 1.8 0.541 3 Monkeys (rhesus) 14.49 0.25 12 3.1 0.324 Marmoset $0.14 \cdot 0.72$ 6.2 0.35 0.06 6 0.162Squirrel monkey 0.600.29-0.97 0.09 7 5.3 0.1897-23 20 Baboon 12 0.60 1.8 0.54120 10.33 0.74 27 1.4 0.730 Micro pig 35 40 25-641.14 1.1 0.946 Mini pig

#### https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4804402/

\*Data obtained from FDA draft guidelines.<sup>[7]</sup> FDA: Food and Drug Administration, HED: Human equivalent dose

#### The table predicts that a mouse can withstand a 12.3 times stronger toxic dose compared to a human;

However, female mice may be strongerothan male mice against drug toxicity.

# Woman dies after accidentally brushing teeth with rat poison

It is fairly common to mistake a specific brand of rat poison for toothpaste due to similar packaging

Kate Ng | Tuesday 26 November 2019 13:04 | comments

#### THE TIMES OF INDIA

City	Bhopal	Mumbai	Delhi	Bengaluru	Hyderabad	Kolkata	Chennai	Agra	Agartala	Ahmedabad	Ajmer	Allahabad	Amaravati	•••	ର୍ ≡
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NEWS / CITY NEWS / BHOPAL NEWS / Man Dies After Consuming Rat Poison Accidentally In Bhopal

1 THIS STORY IS FROM MAY 18, 2019

#### Man dies after consuming rat poison accidentally in Bhopal

TNN / May 18, 2019, 09:35 IST

# Equivalent IVM dose in different animals

- Zelenkov/McCullough protocol for humans 0.5 mg/kg per dose
- Equivalent dose in animals (based on theoretical considerations cited in previous slide)
  - Rats/Mice 3 mg/kg to 6mg/kg per dose.
  - Dogs 1mg/kg per dose.

# Single dose studies

All Merck reports cited are *unpublished and not available in the public domain.* 

The summary of Merck reports were taken from the WHO/FAO report available here

http://www.inchem.org/documents/jecfa/jecmono/v27je03.htm

FDA documents available in link below summarizes clinical trials used for approving lvermectin in the USA.

https://www.accessdata.fda.gov/drugsatfda\_docs/nda/96/050742ap.pdf

All doses were given orally, except where explicitly mentioned.

# Merck study 1979

- Young rats were fed one dose of IVM ranging from 2.5 mg/kg to 96 mg/kg
  - Adverse reactions decreased activity, salivation, abnormally low breathing rate, nervous system disorder, death
  - Merck did not clarify which doses caused which effects.

Zelenkov/Mc Cullough equivalent single dose for rats/mice = 3 mg/kg to 6 mg/kg

### Merck study 1979

- Beagles received one dose of IVM at 2.5, 5.0, or 10 mg/kg
  - Adverse reactions injury to muscles/nerves of eyes, absence of pupil response, nervous system disorder, tremors, vomiting



Zelenkov/Mc Cullough equivalent single dose for dogs = 1 mg/kg

### Pulliam et al., 1985

- Collies received one dose of IVM at 0.05, 0.2 or 0.6 mg/ kg
  - Adverse reactions nervous system damage, paralysis, breathing problems, death

Zelenkov/Mc Cullough equivalent single dose for dogs = 1 mg/kg



## Multiple dose studies

All Merck reports cited are *unpublished and not available in the public domain.* 

The summary of Merck reports were taken from the WHO/FAO report available here

http://www.inchem.org/documents/jecfa/jecmono/v27je03.htm

FDA documents available in link below summarizes clinical trials used for approving Ivermectin in the USA.

https://www.accessdata.fda.gov/drugsatfda\_docs/nda/96/050742ap.pdf

All doses were given orally, except where explicitly mentioned.

# Merck study 1979

- Rat pups (3 to 4 weeks old) received 0.4, 0.8, and 1.6 mg/ kg per day for 14 weeks.
  - Adverse reactions from 8th day enlarged spleen, bone marrow disease

Zelenkov/Mc Cullough equivalent single dose for rats/mice = 3 mg/kg to 6 mg/kg

### Merck study 1979

- Beagles received IVM at 0.5, 1.0, or 2 mg/kg per day up to 12 weeks
  - Adverse reactions injury to muscles/nerves of eyes, absence of pupil response, nervous system disorder, intermittent or constant tremors, eating disorders, weight loss, dehydration.



Zelenkov/Mc Cullough equivalent single dose for dogs = 1 mg/kg

### **Reproduction studies**

- Female rats were dosed at 0.4, 0.8 and 1.6 mg/kg per day 15 days prior to mating and 20 days after child birth.
  - Statistically significant deaths among pups. Before death, pups were hypothermic (Merck, 1979)

Zelenkov/Mc Cullough equivalent single dose for rats/mice = 3 mg/kg to 6 mg/kg

## Embryotoxicity

- Female mice received 0.2, 0.4, 0.8, or 1.6 mg/kg per day from day 6 to 15 of pregnancy.
  - Adverse reactions deaths of female mice, tremors, coma, abortions; birth deformities among baby mice (Merck and co., 1979, 1980)

Zelenkov/Mc Cullough equivalent single dose for rats/mice = 3 mg/kg to 6 mg/kg

### **Biochemical toxicity**

- Qureshi 2013 biochemical effects of IVM on blood plasma and liver tissue of Wistar albino rats.
- intraperitoneal injections with control (saline) and IVM doses of 5, 10, and 15 mg/ kg body weight (single dose)
- IVM led to oxidative damage of liver as indicated by
  - elevated levels of AST, ALT, triglycerides, cholesterol and LDH in blood plasma.
  - elevated levels of Malondialdehyde (MDA) in liver tissue.
  - depleted levels of glutathione in liver tissue.
- In addition, IVM led to genotoxicity as indicated by reduction of DNA and RNA concentration in liver tissue.

#### Intraperitoneal injection



The drugs are injected outside the stomach and other organs, into the peritoneal cavity. https://youtu.be/BOS5oJyncWQ

### Further reading

- Qureshi, Shoeb. "Biochemical toxicity of ivermectin in Wistar albino rats." *Am-Eur J Toxicol Sci* 5.1 (2013): 15-9.
- Ahmed, Ahmed Ezzat, et al. "Vitamin E and selenium administration synergistically mitigates ivermectin and doramectin-induced testicular dysfunction in male Wistar albino rats." *Biomedicine & Pharmacotherapy* 124 (2020): 109841.
- GabAllh, Mahmoud S., et al. "Pathological studies on effects of ivermectin on male and female rabbits." *Benha Veterinary Medical Journal* 32.1 (2017): 104-112.
- Saqib, Muhammad, Ghazanfar Abbas, and Mudassar Niaz Mughal.
  "Successful management of ivermectin-induced blindness in an African lion (Panthera leo) by intravenous administration of a lipid emulsion." *BMC veterinary research* 11.1 (2015): 1-7.

#### In Vitro studies

#### IVM applied to tissue culture of hamster ovary cells



#### In vitro genotoxic and cytotoxic effects of ivermectin and its formulation ivomec<sup>®</sup> on Chinese hamster ovary ( $CHO_{K1}$ ) cells

G. Molinari, S. Soloneski, M.A. Reigosa, M.L. Larramendy\*

**Published 2009** 

Cátedra de Citología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina

### Molinari et al, 2009

- Chinese Hamster ovary cells were cultured for this experiment.
- IVM was then introduced in concentrations usually given to animals (for pest control).
- Positive controls (a substance that is known to create cytotoxic or genotoxic effects) and negative controls (a substance that is not associated with toxic effects) were also used.

#### Cytotoxic effects

Damages to cell functions, cell multiplication etc

#### IVM suppressed activity of lysosomes



Lysosomes remove waste particles from tissues or recycle material Reduction of lysosome activity signifies cell toxicity

# IVM suppressed cell metabolism



At moderate doses IVM suppressed cell metabolism (measured using MTT assay which evaluates mitochondrial activity) stronger than ethanol

#### Mitosis

In humans, nearly 2 Trillion cells divide by mitosis every day

We have about 36 Trillion cells in our body.



Mitosis cell division creates two genetically identical daughter diploid cells. The major steps of mitosis are shown here. (Image by Mysid from Science Primer and National Center for Biotechnology Information)

#### source: https://askabiologist.asu.edu/cell-division

IVM at the higher doses tested (> $25\mu g/ml$ ) reduced mitosis by 75% similar to Bleomycin, a common chemotherapy agent which is known to strongly inhibit cell division. (Table 1 of Molinari et al 2009).

#### Genotoxicity

#### Bleomycin (BLM) is a strong chemotherapeutic agent

#### Table 2

Frequencies of undamaged, slightly damaged and damaged cells in control and ivermectin- and ivomec<sup>®</sup>-treated CHO<sub>K1</sub> cells detected by the electrophoresis assay (SCGE).<sup>a</sup>

	Doses (µg/ml)	Number cells examined	Percentage of cells <sup>b</sup>					
			Undamaged	Slightly damaged	Damaged			
	0.0	150	90.0 ± 3.0	10.0 ± 3.0	0.0 ± 0.0			
→ BLM <sup>c</sup>	1.0	150	$33.0 \pm 2.6^{***}$	$54.0 \pm 1.0^{***}$	$13.0 \pm 1.6^{***}$			
Ivermectin	5.0 25.0 50.0	150 150 150	$\begin{array}{c} 67.0 \pm 5.6^{**} \\ 65.6 \pm 5.4^{**} \\ 63.3 \pm 4.6^{***} \end{array}$	$\begin{array}{c} 30.0 \pm 6.0^{***} \\ 33.7 \pm 5.4^{***} \\ 33.3 \pm 4.5^{***} \end{array}$	$\begin{array}{c} 3.0\pm1.3\\ 0.7\pm0.3\\ 3.3\pm1.2\end{array}$			
Ivomec	5.0 25.0 50.0	150 150 150	$\begin{array}{l} 77.3 \pm 5.4 \\ 74.6 \pm 6.1 \\ 67.0 \pm 0.6^{**} \end{array}$	$\begin{array}{c} 22.6 \pm 5.4^{**} \\ 23.3 \pm 5.5^{**} \\ 29.0 \pm 0.3^{***} \end{array}$	$\begin{array}{c} 0.0 \pm 0.0 \\ 2.0 \pm 0.6 \\ 4.0 \pm 0.6^* \end{array}$			

- Gene damage was assessed in terms of DNA strand breaks.
- Gene damage could not be assessed above 50  $\mu g/ml$  dose, because of cell death above these doses.

*vitro* cytogenetic bioassays. However, our results clearly revealed the induction of geno- and cytotoxicity when mammalian cells are exposed *in vitro* to IVM, at least on CHO<sub>K1</sub> cells. Thus, further investigation/s is/are needed to acquire a comprehensive and exhaustive knowledge of the possible mechanism/s through which IVM and ivomec<sup>®</sup> exert their toxic effects.

IVM toxicity not only kills cells, but it also damages DNA.

Genotoxicity empirically supported by the birth defects among baby rats/mice.



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To the best of our knowledge

no studies on the geno- and cytotoxic effects exerted by IVM have been reported so far, neither for humans as well as for the major vector organisms in which the compound is employed worldwide to prevent parasitosis in their hosts. The Environmental of Protec-

The above paragraph (in a peer reviewed paper) suggests that no one has systematically studied the geno- and cytotoxic effects of Ivermectin on humans, prior to 2009.

Thus, the claims regarding safety of Ivermectin on humans is not supported by scientific studies, *but by hearsay.* 

#### Other issues of concern

- IVM in normal doses caused unusual deaths of nursing home residents when treated for scabies, within 6 months of treatment <u>https://www.jwatch.org/jd19970701000009/1997/07/01/ivermectin-and-death-elderly-patients</u>
  - Sparsa, A., et al. "Systemic adverse reactions with ivermectin treatment of scabies." Annales de Dermatologie et de Venereologie. Vol. 133. No. 10. 2006. <u>https://europepmc.org/article/med/17072195</u>
  - Njoo, F. L., et al. "Neutrophil activation in ivermectin-treated onchocerciasis patients." *Clinical & Experimental Immunology* 94.2 (1993): 330-333., <u>https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1365-2249.1993.tb03452.x</u>
  - Trailović, S. M., and Vladislav M. Varagić. "The effect of ivermectin on convulsions in rats produced by lidocaine and strychnine." *Veterinary Research Communications* 31.7 (2007): 863-872.
- There are some reports on IVM affecting human/animal fertility
  - <u>https://www.scholarsresearchlibrary.com/articles/effects-of-ivermectin-therapy-on-the-sperm-functions-of-nigerian-onchocerciasis-patients.pdf</u>
  - Moreira, N., Maria Martha Bernardi, and Helenice de Souza Spinosa. "Ivermectin reduces sexual behavior in female rats." *Neurotoxicology and teratology* 43 (2014): 33-38.
- Enhanced adverse impacts of IVM when used with calcium channel blockers (often used to treat elevated blood pressure)
  - El-Ashmawy, Ibrahim M., Abeer F. El-Nahas, and Aida E. Bayad. "Teratogenic and cytogenetic effects of ivermectin and its interaction with P-glycoprotein inhibitor." *Research in veterinary science* 90.1 (2011): 116-123.
  - El-Nahas, Abeer F., and Ibrahim M. El-Ashmawy. "Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats." *Environmental toxicology and pharmacology* 26.2 (2008): 206-211.



Antiviral Research Volume 178, June 2020, 104787



#### The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 *in vitro*

Leon Caly <sup>a</sup>, Julian D. Druce <sup>a</sup>, Mike G. Catton <sup>a</sup>, David A. Jans <sup>b</sup>, Kylie M. Wagstaff <sup>b</sup>  $\stackrel{\circ}{\sim}$   $\boxtimes$ 

#### Caly et al 2020 Is this study valid?

This is the study to first claim that IVM inhibits the replication of SARS-CoV-2 virus.



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- Caly et al 2020 claims that cell cultures treated with IVM did not become positive with the RT-PCR test (cycle threshold,  $C_t = 28$  cycles)
- Based on this, they suggest that IVM inhibits the replication of SARS-CoV-2 in vitro.
- Can we accept this claim?

## Caly et al 2020

- A central question is how much IVM did they use and what is the impact of this dose on the cell culture.
  - They used 1ml of  $5\mu M$  to treat 24 wells containing vero/hSLAM cells (kidney cells of African green monkey)
    - 1 well contains  $2.4 \times 10^5$  cells, each weighing 1 nano gram.
    - therefore 24 wells contain  $2.4 \times 10^5 cells \times 1 ng/cell \times 24 wells = 0.00576g$
    - $5\mu M$  of IVM at 1mL is 4.38  $\mu g$  ( $5\mu M \times 1mL \times 875.1g/mol$ )
    - therefore the dose of IVM is  $4.38\mu g/0.00576g = 760 mg/kg$
    - a typical dose given to humans or animals for pesticidal use is 0.2 mg/kg; thus the cell culture dose used in Caly et al 2020 is about 4000 times the pesticidal use.

#### These calculations are copied from Tom Musgrove's response in https://www.quora.com/What-is-the-recommended-dosage-for-lvermectin-for-COVID-19

# Implications of high IVM dose in Caly et al 2020

- Caly et al's IVM dose is 760mg/kg; this is for a 60 kg person a ridiculous 45 g per day.
- More importantly, Molinari et al., 2009 shows that even moderately high, but therapeutic doses of IVM in cell culture leads to cell death (Fig 1, Fig 2, Table 1; also see slide 31).
  - Therefore, one interpretation of Caly et al's PCR test result is that the test became negative because there was no nucleic acid left to amplify.
  - Why would a researcher provide such insane doses to a cell culture is another question that needs to be asked!

### My take

- Ivermectin has been given to millions of poor people in the developing world.
- This does not imply, despite the billions of doses given over 3 decades, that Ivermectin is a safe drug.
- It is assumed to be safe, but this claim is not backed by adequately powered clinical trials in the developed world, or by adverse effects monitoring in the developing world.
- The symptoms associated with Ivermectin toxicity suggest that it adversely affects the Central Nervous System.
- Additionally, there are concerns of genotoxicity as well as concerns related to human fertility.
- Care should be exercised when prescribing Ivermectin as either prophylaxis or as treatment.