Research Exam Q7 – *Biomedical evidence in practice* (2019-2020)

April 8, 2020

During the exam, you have access on a computer to these books:

- Baynes & Dominiczak: Medical Biochemistry
- Campbell: Statistics at square one
- Donders: Literature Measurement errors
- Fletcher: Clinical Epidemiology
- van Oosterom en Oostendorp: Medische Fysica
- Petrie and Sabin: Medical Statistics at a Glance
- Turnpenny: Emery's Elements of Medical Genetics
- Casarett & Doull's Essentials of Toxicology (3e)
- Form with statistical formula's

You are allowed to use a calculator of the type Casio FX-82MS.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Write your name and student number on the first page of each question!

Name:

Student Number:

General question (Gert Olthuis)

Question 1 (6 points)

Decision-making in public health policy is surrounded with uncertainties, both with regard to factual knowledge and with regard to normative issues (ethics). This is also the case for the novel coronavirus outbreak that started in Wuhan, China. The World Health Organization (WHO) published a Strategic Preparedness and Response Plan on the 5th of February to limit further spreading of the virus. The text in the box below outlines the plan and clarifies its objectives. Read this text and answer the question below that deals with normative issues surrounding this WHO plan.

To fight further spread of the new coronavirus (2019-nCoV) outbreak in China and globally, and protect states with weaker health systems, the international community has launched a US$675 million preparedness and response plan covering the months of February through to April 2020.

"My biggest worry is that there are countries today who do not have the systems in place to detect people who have contracted with the virus, even if it were to emerge," said Dr Tedros Adhanom Ghebreyesus, WHO Director-General. "Urgent support is needed to bolster weak health systems to detect, diagnose and care for people with the virus, to prevent further human to human transmission and protect health workers."

The Strategic Preparedness and Response Plan (SPRP) for the new coronavirus lays out activities and resources needed by international health organizations globally, including WHO, to implement priority public health measures in support of countries to prepare and respond to nCoV-2019 for a period February-April 2020. The objectives of the plan are to limit human-to-human transmission of the virus, particularly in countries most vulnerable if they were to face an outbreak; identify, isolate and care for patients early; communicate critical risk and event information; minimize social and economic impact; reduce virus spread from animal sources; and address crucial unknowns.

(taken from www.who.int)

Name two normative issues from the WHO plan in the box and explain them with reference to the text in the box (each issue + explanation = 3 pnts).

Solidarity: rich countries help countries with weaker health systems

Social justice: allocation of scarce resources to help countries with weak healthcare systems

(Collective) responsibility: fighting the outbreak of the virus is a global, and collective responsibility, not the responsibility of individual countries

Prevention of harm: further transmission would harm a lot of people, especially in poorer countries with weaker health systems. Early detection is needed to prevent further spread of virus

Freedom ; Isolation of patients implies a serious limitation of the freedom (or individual autonomy) to move around.

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Vaccination (Saskia van Selm)

Question 2 (9 points)

Currently, a coronavirus outbreak is ongoing worldwide and it is questionable if actions that are taken can stop further spread of the virus. Since the DNA sequence of this new coronavirus was published, multiple companies have started the development of a vaccine against this virus.

a. This vaccine can be a recombinant subunit vaccine. Why would this be a suitable vaccine type? What type of immunity will be aimed to be induced by this vaccine? (3 points)

(Easy to produce OR safe; (neutralizing) antibodies)

b. After several developmental stages and animal testing, the vaccine will be tested in humans. After Phase I studies for reactogenicity and immunogenicity, Phase II and Phase III will be started to establish safety and efficacy. How is efficacy established in Phase III studies? Explain in a few sentences how this works. (3 points)

(Randomized Controlled Trial: Compare incidence of disease between a vaccinated group and a placebo (vaccinated) group)

c. Please elaborate on an ethical reason why it will be hard to perform standard efficacy studies for vaccines if mortality caused by the coronavirus infections is relatively high? (3 points)

(a placebo group will be not ethical, everybody wants the vaccine)

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Vaccination (Saskia van Selm)

Question 3 (8 points)

The Dutch Health Council advices the minister on the introduction of new vaccines in the Dutch national program. They use four main criteria for labelling a vaccine as collectively essential. You get the following information on a new vaccine against shingles (gordelroos):

Shingles will be developed by 1 out of 3 people during their life. Who had chickenpox (waterpokken), are at risk for shingles. The risk of getting shingles and having serious complications increases with age. About 10% of patients who develop shingles develop nerve pain that lasts for months or years after the rash disappears. This is called postherpetic neuralgia (PHN). Shingles may lead to other serious complications involving the eye, including blindness. Rarely it can also lead to pneumonia, encephalitis or death. Shingles vaccination is the only way to protect against shingles and PHN, the most common complication from shingles. Centers for Disease Control recommends that healthy adults 50 years and older get two doses of the shingles vaccine called Shingrix (recombinant zoster vaccine), separated by 2 to 6 months, to prevent shingles and the complications from the disease. Your doctor or pharmacist can give you Shingrix as a shot in your upper arm.
Shingrix provides strong protection against shingles and PHN. Two doses of Shingrix is more than 90% effective at preventing shingles and PHN. Protection stays above 85% for at least the first four years after you get vaccinated.

a. For two of the main criteria for the inclusion of a vaccination in the public program, information is given in the text above. Describe the information given for these 2 criteria. Reflect on whether these criteria are met for labelling a vaccine as collectively essential. Explain your answer. (4 points)

b. Information is missing in the text above in order to evaluate whether a vaccine is suitable for introduction in the national program for the other two main criteria. What are the missing criteria? Please explain their importance. (4 points)

A Disease burden, effectiveness
B safety, efficiency

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Risk assessment (Frans Russel)

Question 4 (15 points)

Tebuconazole (TEB) is a widely used triazole fungicide that is listed as a possible endocrine-disrupting agent. The acceptable daily intake (ADI) is 0.03 mg/kg body weight. Humans may be exposed via the consumption of fruits, vegetables, grains, and drinks (e.g., wine) that could contain residues of TEB. Residents living close to agricultural land may additionally be exposed via inhalation or direct skin contact during spray applications of TEB.

A human volunteer study was performed with the aim to describe the time course of urinary excretion after controlled oral and dermal exposure of TEB. Healthy volunteers received on separate occasions a single oral dose of 1.5 mg of TEB and a single dermal dose of 2.5 mg during 1 h. Complete urine voids were collected over 48 h post-administration and the main metabolite hydroxytebuconazole (TEB-OH) was quantified in each urine sample. The following toxicokinetic results (mean ± SD) were obtained.

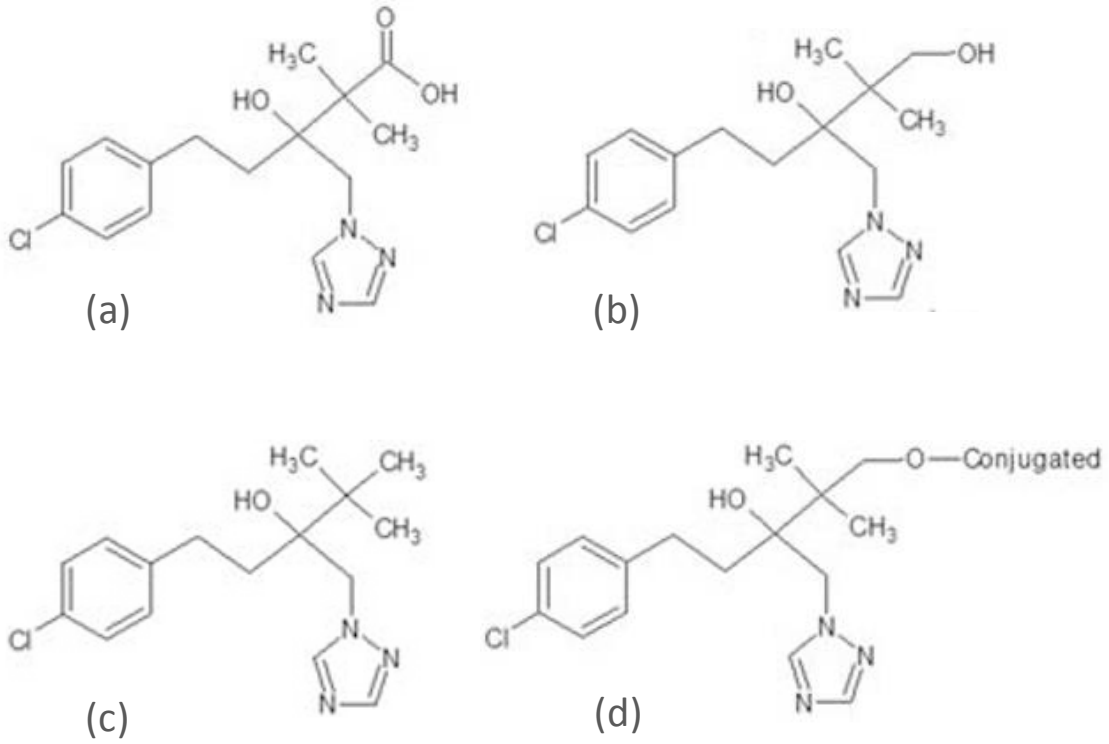
	Oral (TEB 1.5 mg)	Dermal (TEB 2.5 mg, 1h)
TEB-OH excreted (µg/48h)	520 ± 150	26 ± 12
$t_{1/2}$ (h)	7.8 ± 1.0	15.8 ± 3.3
T_{max} (h)	1.4 ± 0.7	20.8 ± 6.7
C_{max} (µmol/L)	2.4 ± 1.7	0.07 ± 0.04

a. Explain the difference in $t_{1/2}$ of TEB-OH between oral and dermal exposure. (3 points)

b. Explain the difference in urinary excretion of TEB-OH between oral and dermal exposure. (3 points)

c. In the urine collected over 48h of a person weighing 70 kg, 900 µg TEB-OH was measured. What is the conclusion about the intake of TEB by this person and whether it was above the ADI. Explain your answer. (4 points)

TEB is converted into its main biotransformation product TEB-OH. In the figure below TEB and its metabolites are listed.



d. Which of these molecules is the parent compound (TEB)? (1 point)

e. Via which biotransformation reaction is TEB converted into TEB-OH? (2 point)

f. The conjugate in molecule (d) is a sulfate or glucuronide. From which of the molecules (a, b or c) is this conjugate formed? (2 point)

Answers

a. *The relatively slow process of dermal absorption of TEB as compared to oral absorption (rate-limiting step in the elimination ($t_{1/2}$) of TEB-OH).*
b. *The bioavailability of TEB is much lower after dermal absorption, because the skin acts as a reservoir and is much less permeable as the intestinal wall.*
c. *As the route of exposure (oral or dermal) is not known a precise estimation of the intake is not possible. Suppose the exposure was exclusively via food (best-case scenario) than the fractional excretion after oral exposure in the human volunteer study (0.52 mg/1.5 mg = 0.35) can be used to calculate the intake: 0.9 mg/0.35 = 2.6 mg (ADI = 0.03 mg/kg*70 kg = 2.1 mg. This means that the intake exceeded the ADI. If there was also skin absorption (fractional excretion 0.01) of TEB, then the intake must have been much higher.*
d. *(c)*
e. *Hydroxylation or oxidation = 2 points, Phase I reaction = 1 point*
f. *(b), it is an ether conjugate (sulfate or glucuronide) that is typically formed with a hydroxyl group.*

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Risk assessment (Frans Russel)

Question 5 (10 points)

Brain damage as a result of recreational use of nitrous oxide?

“If you've taken a night out in East London's Shoreditch area during the past year, you'd be forgiven for believing that the latest must-have hipster accessory was a blown-up balloon. You'd be...half right. Rather than being filled with helium, they're filled with Nitrous Oxide (otherwise known as laughing gas), a "legal high" that's all the rage with party people in the area at the moment.

Nitrous Oxide, though relatively harmless for many in small doses, can occasionally prove fatal when inhaled, and can lead to anaemia, bone marrow suppression and central nervous system poisoning.” (Source: gizmodo.co.uk)

Read the abstract of a rat toxicity study (below) and formulate the answers to the questions.

Prolonged Exposure to Inhalational Anesthetic Nitrous Oxide Kills Neurons in Adult Rat Brain

V Jevtovic-Todorovic 1, J Beals, N Benshoff, J W Olney

Short-term exposure of adult rats to nitrous oxide (N2O), an inhalational anesthetic and NMDA (N-methyl-D-aspartate) antagonist, causes a reversible neurotoxic vacuole reaction in neurons of the posterior cingulate/retrosplenial cortex (PC/RSC) which resembles that caused by low doses of other NMDA antagonists. Since high doses or prolonged exposure to other NMDA antagonists can cause neurons to die, we assessed whether prolonged N_2O exposure might also cause neuronal cell death.

Adult female Sprague-Dawley rats were exposed to 150-vol% N2O (approximately EC50 for N_2O anesthesia in rats) for various durations from 1 to 16 h. The time course for onset and disappearance of the reversible vacuole reaction was studied, as was the time course and dose requirement for triggering cell death. A maximum vacuole reaction was observed in PC/RSC neurons in brains examined immediately after 3 h of 150-vol% N_2O exposure and the same magnitude of vacuole reaction was observed when brains were examined immediately after a longer period of N_2O exposure. When N2O was terminated at 3 h and the rats were killed 1 h later, the vacuole reaction was markedly diminished and if the rats were killed 3 h later the vacuole reaction had completely disappeared. Prolonged exposure to 150-vol% N_2O (for 8 h or more) caused neuronal cell death which was detectable by silver staining 32 h later. Concurrently administered GABAergic agents, diazepam (an i.v. anesthetic), or isoflurane (an inhalational anesthetic), prevented this cell death reaction.

Our findings demonstrate that short-term exposure of adult rats to N2O causes injury to PC/RSC neurons that is rapidly reversible, and prolonged N_2O exposure causes neuronal cell death. These neurotoxic effects, including the cell death reaction, can be prevented by coadministration of GABA-mimetic anesthetic agents. Duration of NMDA receptor blockade appears to be an important determinant of whether neurons are reversibly injured or are driven to cell death by an NMDA antagonist drug.

Source: Neuroscience. 2003;122(3):609–616.

a. Is the study by Jevtovic-Todorovic relevant to the potential health risk of recreational use of N_2O referred to in the post on the gizmodo website? Motivate your answer. (3 points)

b. In this study the exposure is expressed in units of time (hours). Explain how this can be used as a dose unit in a risk assessment. (2 points)

c. What are the uncertainties for the use of these animal data for the risk assessment of the recreational use of N_2O? (2 points)

d. Calculate a no observed effect level (NOAE) to estimate a safe duration for inhalation of N_2O in minutes to prevent irreversible brain damage in humans using toxicity data from the abstract. (3 points)

Answers

a. Yes, the animal study is an appropriate experimental set-up to evaluate the inhalation toxicity of these high doses of pure N_2O to the brain. The endpoint studied is reversible and irreversible damage to the brain at a cellular level, which is a relevant effect to evaluate potential damage to the central nervous system in humans.
b. In toxicology, dose can be expressed as concentration x time. The most common method is to increase concentration and take the exposure duration as the constant. A simpler way that is relevant to the recreational use of N_2O is to study (only) exposure duration.
c. When using animal data to estimate health risk in humans the two most important sources of uncertainty are intraspecies differences and interspecies differences.
d. Similar to the use of concentration units for dose when using exposure duration. The exposure duration that did not results in irreversible brain damage is 3 h (complete recovery of the vacuole reaction occurred within 3 h). For extrapolation to humans this exposure duration is divided by a factor 100 to arrive at 3/100= 0.03 h x 60 min = 1.8 min as the time corresponding to a NOAEL. So, based on the available rodent study a tentative safe duration for inhaling high levels of N_2O would be 1.8 min = 108 sec.

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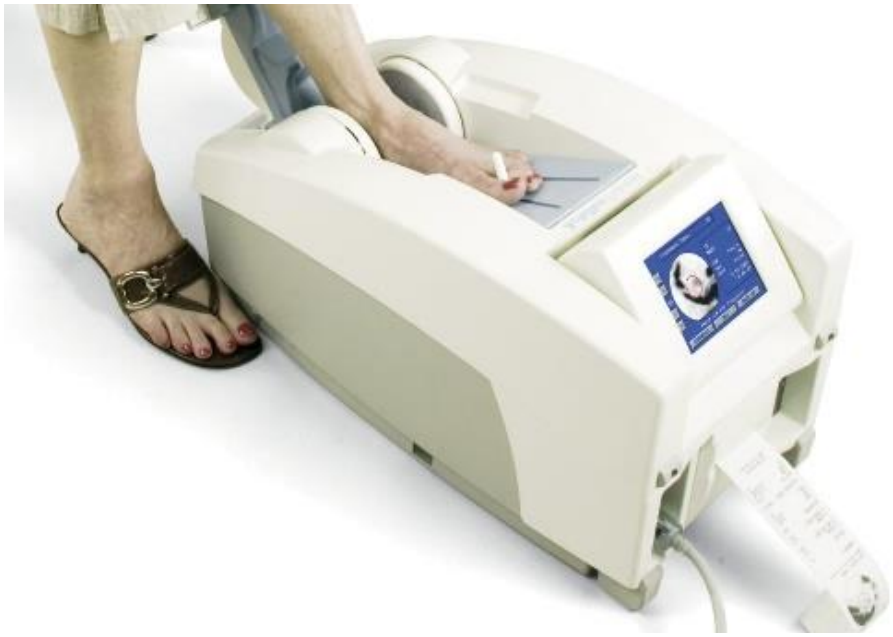
Screening as early diagnostics (Jos van Dijck)

Question 6 (12 points)

Quantitative ultrasound (QUS) for osteoporosis screening is potentially interesting because it predicts fracture risk, is portable and relatively inexpensive.



Ultrasound imaging of the heel (USH) is a much simpler test to identify osteoporosis than the standard test, dual-energy x-ray absorption (DEXA). A study was performed in which 1945 women from the general population screened and evaluated for osteoporosis with USH and compared to the reference test DEXA. The results are presented in Table 6.1.

Table 6.1 –Outcomes of screening for osteoporosis with USH: a cross-sectional study.

	Osteoporosis (DEXA)		
USH test	present	absent	total
positive	338	637	975
negative	90	880	970
Total	428	1517	1945

a. Calculate the four basic screening test properties (%) of USH: sensitivity, specificity, positive predictive value (PV+) and negative predictive value (PV-). (2 points)
SENS = 338/428 = 79%; SPEC = 880/1517 = 58%; PV+ = 338/975 = 35%; PV- = 880/970 = 91%

b. Calculate the likelihood ratio of a positive test (LR+). (1 point)
LR+ = sens / (100-spec) - 79% / 42% = 1,9

c. For good communication and decisions whether or not to screen with USH, what performance measures of the screening tool are preferable: sensitivity and specificity rates, or post-test probabilities (PV+ and PV-) of having osteoporosis? Explain your answer. (2 points)

Uitleggen wat SENS resp. SPEC voor de individuele persoon betekent is lastig, ook als je dat doet in de complementaire termen i.c. %FN en %FP.
Duiding a.d.h.v. voorspellende waardes gaat beter omdat die iets zeggen over de kans om de daadwerkelijk de aandoening te hebben bij een concrete testuitslag. Daar wordt ook in meegenomen de voorafkans op de aandoening in de onderzochte populatie.

d. Based on the quantitative figures from a and b, is the USH test a meaningful screening tool to detect osteoporosis? Explain your answer. (3 points)

LR+ van 1,9 is heel aardig voor een diagnostische test, maar voor een screeningstest gaat de voorkeur sterk uit naar een discriminerend vermogen van >20, anders speelt het % FP te zeer op.
Oordelend naar voorspellende waarde zou zowel de PV+ als de PV- bij voorkeur richting 100% moeten gaan. Dat is nu zeker niet het geval voor PV+, wel de PV-, maar dit laatste betekent altijd nog dat van de mensen met een negatieve screeningstestuitslag, doorgaans de meerderheid van de gescreenden, toch nog altijd 9% een FN uitslag heeft.

e. It is argued that this screening tool has poor performance because of its high rate of false positive outcome necessitating additional evaluation at high medical costs. If a different cut-off level of USH test positivity is used ─ higher or lower, respectively ─ what will happen to the false-positive rate of the screening test? Explain your answer. (4 points)

Het afkappunt voor testpositiviteit bij een hogere kwantitatieve echoscore leggen betekent dat minder mensen met osteoporose testpositief zullen zijn: de SENS wordt kleiner. Van de andere kant zullen meer mensen zonder de aandoening een FP testuitslag krijgen: de SPEC verbetert.
Het omgekeerde treedt op als het afkappunt bij een lagere kwantitatieve echoscore komt te liggen.