Research Exam Q7 – *Biomedical evidence in practice* (2020-2021)

April 7, 2021

NOTE ON SCIENTIFIC INTEGRITY:

'By taking the exam, the student declares that no plagiarism is or will be committed. If the lecturer has the suspicion that fraud has been committed, the student will be contacted. If needed, the case will be redirected to the Examination Board.'

- ***We expect you to take this exam individually, without consulting fellow students or others***
- ***If you use sources, refer to them in Vancouver style.***
- ***If you quote from the sources you consult, reduce this to a minimum and be precise and careful***

Do not use this document with questions as an answer sheet. Please write down your answers in a separate Word-document.

- Start of the exam: 13.00 hrs.
- Write your name and student number in 'koptekst' so each page contains this information.
- Begin the answer of a question on a new page.
- The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.
- This is an open book exam. *Essentials of Toxicology* is the only book you might need. A pdf of the first 10 chapters of this book is provided on Brightspace.
- You are allowed to use a calculator of the type Casio FX-82MS.
- **Upload this Word-document via Brightspace, deadline 15.30 hrs on 7 April.** Urkund will be used to check plagiarism.
- **Also send the Word-document with answers by e-mail (with your personal student-account!) to BMWQ7.RHA@radboudumc.nl**
- Coordinator Gert Olthuis can be reached during the exam: gert.olthuis@radboudumc.nl.

General question (Gert Olthuis)

Question 1 (6 points)

The Health Council provides advice to the government regarding public health matters. This involves complex decision making based on factual and normative (un)certainties.

The text in the box below is taken from the work programme which the Health Council recently published (2021). It presents the intention to advise on incidental findings from screening programmes.

Incidental findings from population screening programmes

In screening programmes and health checks, the use of imaging techniques and DNA testing can result in findings that are unrelated to the primary focus of the screening programme in question. In practice, these incidental findings can lead to situations that involve difficult decisions, in which the participants 'right to know', as well as their 'the right not to know' may be at stake. The Council intends to formulate a set of guiding principles on how incidental findings in population screening programmes should be handled. The details will be published as an unsolicited advisory report. These principles will also be used to assess programmes of this kind.

Taken from: healthcouncil.nl

The table below allows the classification of public health problems, based on certainty pertaining normative criteria and the knowledge about the underlying public health issue.

	HIGH certainty on knowledge	LOW certainty on knowledge
HIGH consensus on normative criteria	A	B
LOW consensus on normative criteria	C	D

Which cell (A, B, C or D) of this table provides the best classification of the advice of the Health Council concerning the occurrence of incidental findings in screening programmes?

Explain your answer with one argument regarding the certainty of knowledge and one argument regarding the consensus on normative criteria surrounding this issue.

Correct cell (2 pt): C

Argument on normative criteria (2 pt): The HC intends to formulate a set of guiding principles on how incidental findings should be handled. This is necessary since there is a low consensus on how to deal with these findings since participants have the 'right to know' and 'the right not to know'.

Argument on knowledge (2 pt): The central question here is: what will happen if particular measures - such as screening programmes - will be introduced. There is certainty screening programmes will result in incidental findings; findings that are un related to the primary focus of screening. We also know that this leads to complex decisions for those involved, both participants and health care professionals. The answer to the question how to deal with this issue however, depends on how to deal with normative uncertainties.

Vaccination (Saskia van Selm)

Question 2 (9 points)

Read the following abstract from an article in *The New England Journal of Medicine*, February 24 (2021):

BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting

BACKGROUND
As mass vaccination campaigns against coronavirus disease 2019 (Covid-19) commence worldwide, vaccine effectiveness needs to be assessed for a range of outcomes across diverse populations. In this study, data from Israel's largest health care organization were used to evaluate the effectiveness of the BNT162b2 mRNA vaccine.

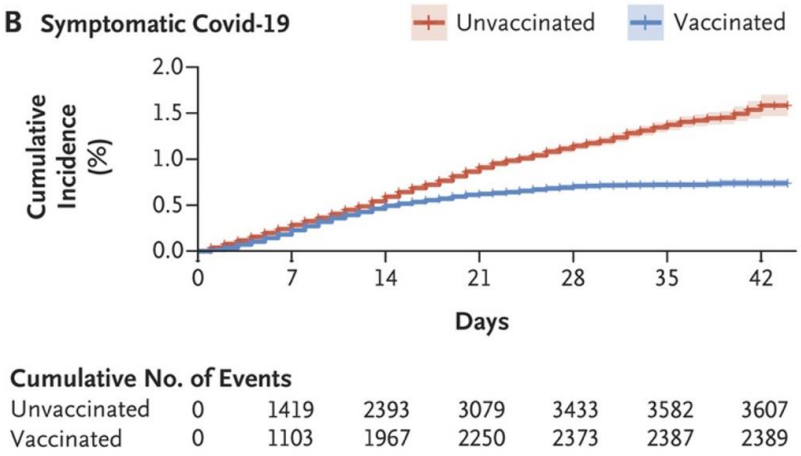
METHODS
All persons who were newly vaccinated during the period from December 20, 2020, to February 1, 2021, were matched to unvaccinated controls in a 1:1 ratio according to demographic and clinical characteristics. Study outcomes included documented infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), symptomatic Covid-19, Covid-19–related hospitalization, severe illness, and death. We estimated vaccine effectiveness for each outcome as one minus the risk ratio, using the Kaplan–Meier estimator.

RESULTS
Each study group included 596,618 persons. Estimated vaccine effectiveness for the study outcomes at days 14 through 20 after the first dose and at 7 or more days after the second dose was as follows: for documented infection, 46% (95% confidence interval [CI], 40 to 51) and 92% (95% CI, 88 to 95); for symptomatic Covid-19, 57% (95% CI, 50 to 63) and 94% (95% CI, 87 to 98); for hospitalization, 74% (95% CI, 56 to 86) and 87% (95% CI, 55 to 100); and for severe disease, 62% (95% CI, 39 to 80) and 92% (95% CI, 75 to 100), respectively. Estimated effectiveness in preventing death from Covid-19 was 72% (95% CI, 19 to 100) for days 14 through 20 after the first dose.

CONCLUSIONS
This study in a nationwide mass vaccination setting suggests that the BNT162b2 mRNA vaccine is effective for a wide range of Covid-19–related outcomes, a finding consistent with that of the randomized trial.

a. The effectiveness established was consistent to the efficacy determined in the phase III clinical trial before the vaccine was marketed. This is very good news, because it is better than expected. Explain why the effectiveness was expected to differ from the phase III clinical trial. (2 points)

No controlled setting: no perfect cold chain, different age distribution, specific risk-groups included that were excluded from the trail, other strains circulating



Cumulative No. of Events							
Unvaccinated	0	1419	2393	3079	3433	3582	3607
Vaccinated	0	1103	1967	2250	2373	2387	2389

Figure 2.1 – Number of events

b. In the numbers below Figure 2.1 the number of events (positive PCR test) in each of the study groups for symptomatic Covid-19 are listed. An important milestone of this vaccine is that it is highly protective against disease. The study was not designed to test viral transmission and infection of persons who did not show any symptoms of infection. Why is knowledge on transmission of the virus after vaccination also very important? (3 points)

Can vaccinated people who do not show any signs of disease still infect others (non-vaccinated individuals): control the Pandemic, stop the spread of the virus, protection non-immune, implications for measurements as social distancing, wearing masks, lockdowns.

c. What should be added to the study design to be able to get information on transmission? (1 point)

Data on asymptomatic infections, you will have to test everybody (or subgroups) on a regular basis.

d. You would like to study vaccine-induced correlates of protection within the vaccination campaign. (3 points)
1: Which samples do you want to study (specify timepoint and kind of sample)?
2: Which specific information is essential to collect from the patients in order to be able to establish a correlate of protection?

3: Of which subgroups do you want to compare the samples? Explain your choice for the subgroups .

1. Blood samples before and after vaccination. 2. Data on the development of disease (and proof of covid-19 infection) 3. Vaccinated people who got the disease and vaccinated people who did not get the disease.

Vaccination (Saskia van Selm)

Question 3 (6 points)

As a vaccine policymaker working for the government, you are invited to provide your view in an item about vaccinations. One of the members sitting at the table is a member of a foundation that has doubts on vaccination. The discussion is about Measles vaccination. Your employer has concerns about the effect of the arguments of people against vaccination on the vaccination coverage and asks you specifically to give a substantive response to their arguments.

a. The critic starts with doubts about the need for introduction of the measles vaccine in 1976: 'At this time complications were rare and mortality was almost zero.. An infection with measles is not dangerous or fatal, on the contrary. Patients recover without medical interventions from most childhood infections. In exceptional situations or cases, measles complications can occur. Only in an extreme case, a child can die from complications.' Please bring forward an important argument to explain the need for measles vaccination. (2 points)
(*Still high incidence of disease, maybe for most self-limiting, but not for everybody, we also want to prevent these*)

b. A pediatrician states that after vaccination with live attenuated viruses, replication (multiplication of the viruses) always takes place on a limited scale. In 5-10% of the vaccinated children, this replication leads to minor symptoms (fever, conjunctivitis and rash). These symptoms are similar to those of a wild type measles virus infection and can occur 5-14 days after vaccination. The critic in vaccination asks: 'Is it possible that the (unintended) effect of injecting measles viruses 'en masse' will result in us creating more (vaccine-induced) measles than we prevent?' Describe two advantages of using a live vaccine above an infection in the case of Measles. Please explain your answer. (2 points)
(*Less severe, positive: it resembles natural infection most compared to other vaccines, so gives good protection*)

c. The critic also wonders if the vaccine is effective. 'Since the introduction of the measles vaccine, approximately 16,000 measles cases have been reported in the past 40 years. This may be a significant underreporting of the number of cases.

According to the RIVM, for example, the actual number of measles cases during the 'epidemic' of 1999/2000 was estimated to be 10 times greater than the number of reported cases. All of these measles reports occurred at a time when vaccination coverage was very high (over 90%). Only in the last three years has there been an official "slight decrease" of about 0.5% per year in vaccination coverage.
Please describe how you can explain these cases during the years and how this relates to the effectiveness of the vaccine. (2 points)
(*Not high enough to protect everybody, coverage not uniform, vaccine is protective, but coverage should be high enough to reach herd-immunity in all communities*).

Risk assessment (Frans Russel)

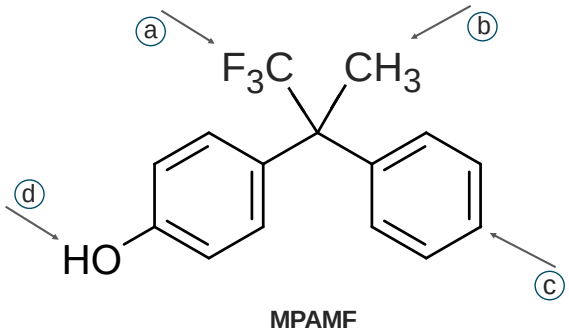
Question 4 (10 points)

The exposure to MPAMF, a compound related to chemicals used in the production of plastics, was investigated after intravenous, oromucosal (under the tongue) and oral administration in Beagle dogs. Values obtained for C_{max} (maximal plasma concentration), T_{max} (time of maximal concentration), AUC (area under the curve) and elimination half-life ($T_{1/2}$) are presented in Table 1.

Table 1. Toxicokinetics of MPAMF after intravenous (i.v.) and oromucosal (o.m.) administration of 5 mg/kg, and oral (p.o.) dose of 20 mg/kg in Beagle dogs.

Parameter	**i.v.** **D = 5 mg/kg**	**o.m.** **D = 5 mg/kg**	**p.o.** **D = 20 mg/kg**
C_{max} (µg/ml)	7.3	6.4	47
T_{max} (min)	3	13	20
AUC (µg*ml/min)	221	145	6
$T_{1/2}$ (min)	48	50	78

a. Calculate the clearance (CL) and volume of distribution (V) of MPAMF. (2 points)
b. Calculate the bioavailability (F) of MPAMF after oromucosal and oral administration, and explain the difference. (2 points)
c. Will MPAMF accumulate in the body of a person that is chronically exposed via food? Motivate your answer. (2 points)
d. In humans, MPAMF is mainly cleared by biotransformation into a glucuronide metabolite. What is the most likely site of glucuronidation in the MPAMF molecule (see structure below): a, b, c, or d? Motivate your answer. (2 points)



MPAMF

e. In some animal species MPAMF undergoes oxidative biotransformation. What is the most likely site for oxidation: a, b, c, or d? Motivate your answer. (2 points)

Anwers

a. After i.v. administration: CL = D/AUC = 5 mg/kg / 221 µg*ml/min = 22.6 (ml/min)/kg. V = T1/2*CL/ln2 = 48 min*22.6 ml/min/kg/0.693 = 1565 ml/kg
b. $F_{o.m.} = AUC_{o.m.}/AUC_{i.v.} = 145/221 = 0.66$ (66 %)
   $F_{p.o.} = AUC_{p.o.}/AUC_{i.v.} * D_{i.v.}/D_{p.o.} = 6/221 * 5/20 = 0.0068$ (0,68 %)
   F after oral administration is much lower, which can be explained by first-pass metabolism in the liver. MPAMF absorbed via the mucous membrane of the mouth enters the general circulation directly and thus escapes from the first-pass effect in the liver.
c. This is very unlikely because of the short half-life.
d. Glucuronide conjugation favours functional polar groups like R-OH, R-COOH, or $R-NH_2$. For MPAMF this will be conjugation to the phenolic OH-group (d)
e. CH3 group (b), because CYP enzymes favour oxidation of an aliphatic group over an aromatic group and over a bulkier and more stable CF_3 group.

Question 5 (10 points)

Inhalation anaesthetics such as nitrous oxide (N_2O), isoflurane and sevoflurane are commonly used in medical, paramedical, and veterinary practice. Since the mid 1950's, concerns have been raised regarding occupational exposure to inhalation anaesthetics.

Previous evaluations of the risk of inhalation anaesthetics resulted in the classification as possible risk factors for adverse reproductive health outcomes based on animal studies. Human data were deemed inadequate primarily because of simultaneous co-exposures to other risk factors for adverse reproductive and developmental outcomes.

Table 1: Study characteristics of studies in female Wistar rats of nitrous oxide (N_2O) and its effect litter size.

Article ID	**Exposure period (gestation days)**	**Exposure pattern**	**N/dose group**	**Air concentration (mg/m^3)**
Vieira et al. (1980)	3 weeks (1-21)	Continuous	12	0
			12	449.1
			12	898.2
			12	1796.5
Vieira et al. (1983)	3 weeks (1-21)	Intermittent 6h/day, 5 days/week	12	0
			12	367.4
			12	734.9
			12	1469.8
			12	7348.9

Here we perform a risk assessment based on available animal evidence on N_2O reproductive and developmental outcomes to inform a health-based recommended occupational exposure limit (OEL) for N_2O with a benchmark dose-response modelling (BMD) approach. Two studies were selected (see Table 1) and used for dose-response modelling (Figure 1 and 2).

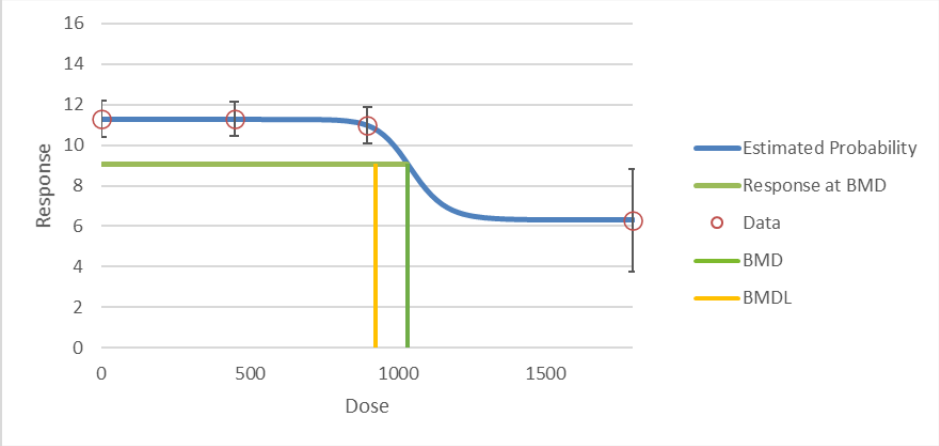


Figure 1. Frequentist Hill model with BMR of one standard deviation for the BMD and 0.95 lower confidence limit for the BMDL (expressed in mg/m^3) for data from Vieira et al (1980) for the endpoint "live foetuses/dam".

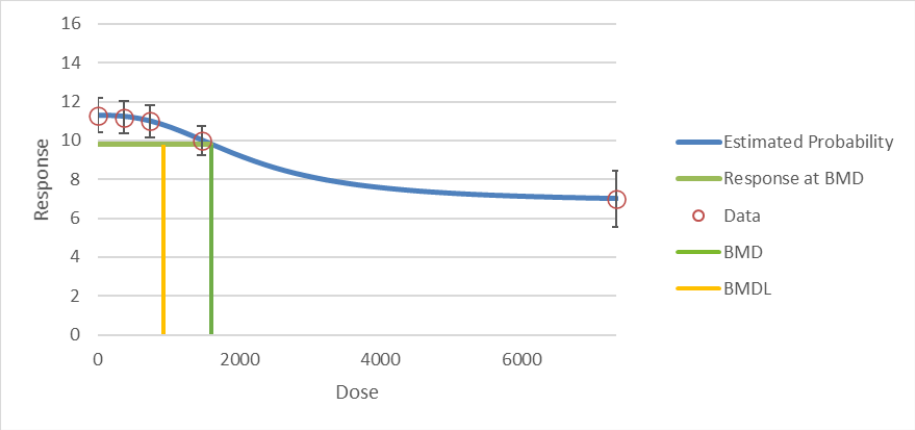


Figure 2. Frequentist Hill model with BMR of one standard deviation for the BMD and 0.95 lower confidence limit for the BMDL (expressed in mg/m^3) for data from Vieira et al (1983) for the endpoint "live foetuses/dam".

Table 2: BMD input and modelling results.

Study	**BMR[a]**	**BMD in mg/m^3**	**BMDL in mg/m^3**
Vieira et al. (1980)	1.4	1,032	924
Vieira et al. (1983)	1.4	1,603	931

[a]*Benchmark response defined as reduction in litter size equivalent to one standard deviation of the average litter size observed in the control group in that study.*

a. Inhalation anaesthetics are often combined. How can this be addressed in the hazard identification? Is it possible to study a mixture of two or three inhalation anaesthetics? Please motivate your answer (1 point)

b. Provide a list of 3 factors to consider for modelling of the exposure using e.g. ConsExpo. Provide a unit for each factor (1.5 points)
c. What is the definition of the 'dose' and how is the 'response' defined in Figures 1 and 2? (1.5 points)
d. The BMR is defined as one standard deviation of the average response observed in the control group. What is the rationale of using this basis for the BMR? (2 points)
e. Which of the two datasets would you prefer to use to derive an occupational exposure limit for healthcare workers who work 40 hour/week in the operation room? Motivate your answer by providing two advantages for the dataset you consider the best (2 points)
f. Calculate the safe level for this endpoint for an 8-hour shift for workers based on the dataset of Vieira et al., 1983. Describe each step in the calculation (2 points)

Anwers

a. Yes, there are two options: complete the risk assessment process for each of the individual substances or as an alternative, prepare a risk assessment for the mixture of the substances which is the better option because the toxicity a mixture can be different than the sum of its components. It is then important to define the exact composition of the mixture.
b. The following factors should be considered as input: weight of substance used (grams), room volume (m3), air exchange rate (per hour)

c. Dose is defined as the air concentration of N_2O in mg/m3; response is defined as the number of live fetuses/dam (no unit)

d. The BMR is defined as additional risk equivalent to the normal variability in unexposed rats, i.e. control group.
e. The dataset of Vieira et al., 1983 has two advantages: (1) The exposure regime closely mimics an intermediate pattern of a work-shift followed by an off-work period (as opposed to the alternative regime which is a continuous exposure). (2) At the point of the BMD the curve shape is well supported by data. This is much less the case for the data in Vieira et al. 1980.
f. The following steps can be taken for Vieira et al., 1983:
   - Extrapolate from 6 to 8 hours: multiply by a factor 6/8 = 0.75
   - Take into account interspecies differences by a factor of 10
   - Take into account intraspecies differences by a factor of 10
   - The conversion then reads: BMDL x 0.75 / 100 = 931 x 0.0075 = 7 mg/m3

Screening as early diagnostics (Jos van Dijck)

Question 6 (11 points)

Major depression is an important health problem with an estimated prevalence of 5-10% in primary care settings. If a practice has sufficient resources for follow-up and treatment of depression, the United States Preventive Services Task Force (USPST) recommends routine screening of adults for major depression, if the condition has not already been identified by a health care professional.

Patient Health Questionnaire 9 (PHQ-9) is a screening instrument to detect major depression. The questionnaire consists of 9 questions (e.g., 'over the past 2 weeks, how often have you been bothered by little interest or pleasure in doing things?'). The answers to the questions sum up to a score between 1 and 27 points. Scores of 10 points or higher are considered indicative of major depression.

A recent individual participant data meta-analysis reported on the diagnostic accuracy of PHQ-9 in primary care setting, pooling the results of studies that compared PHQ-9 to a semi-structured interview by health care professionals as a reference standard. Table 1 shows the results of PHQ-9 in relation to this reference standard for 6725 patients from 29 studies.

Table 1 – Outcomes on screening for major depression by PHQ-9 questionnaire in relation to the reference standard semi-structured interview

	Major depression according to semi-structured interview		
PHQ-9 score	Present	Absent	Total
Positive (≥10 points)	813	870	1683
Negative (<10 points)	111	4931	5042
Total	924	5801	6725

a. Calculate the four basic properties of a screening test (sensitivity, specificity, positive predictive value, negative predictive value) for PHQ-9, based on Table 1. Show your calculations. (4 points)

Sensitivity = 813/924 = 88.0%

Specificity = 4931/5801 = 85.0%

PPV = 813/1683 = 48.3%

NPV = 4931/5042 = 97.8%

The prevalence of major depression among the 6725 study participants was approximately 14%. The prevalence of major depression in general practice is estimated to be lower (5-10%).

b. Describe what happens to the positive and negative predictive values, when you apply PHQ-9 in a population with a lower prevalence of major depression than in the study population. (2 points)

Applying PHQ-9 in a population with a lower pre-test probability will lead to a lower PPV and higher NPV, compared to the study population.

The investigators also assessed which cut-off point of the PHQ-9 score will yield the highest diagnostic accuracy.

c. Describe what would happen to sensitivity and specificity if you increase the cut-off point for test positivity. (2 points)

Increasing the cut-off point will lead to more false negatives and less false positives. By definition, sensitivity will decrease, and specificity increase.

International guidelines do not yet agree on recommending routine screening for major depression in adults. The Canadian Task Force on Preventive Health Care as well as the United Kingdom National Screening Committee recommend against routine depression screening. Based on the data that is currently available, they have concerns that the harms of screening for major depression do not outweigh the benefits.

d. Name three of such harms a screening programme for major depression may have. Your answers may come from the individual or population perspective. (3 points)

- Screening may lead to overdiagnosis/treatment if major depression that is detected earlier or in addition by screening would not need treatment/resolve on its own.

- Being labelled as having a mental health disorder may lead to a social stigma.

- Sustaining adverse effects from antidepressants may be harmful.

- Given the (low) prevalence of major depression and high false-positive rate of PHQ-9 a large recall rate may be expected. Mental health services may not be able to deal with screening, follow-up and management of large numbers of patients.

- The screening programme may not be cost-effective.