Anglo-Chinese Junior College

JC2 Biology Preliminary Examination
Higher 2

A Methodist Institution
(Founded 1886)

CANDIDATE NAME		FORM CLASS	
TUTORIAL CLASS		INDEX NUMBER	

BIOLOGY **9744/03**

Paper 3 Long Structured and Free-response Questions 29 August 2023
2 hours

Candidates answer on the Question Paper.
Additional Materials: Writing paper(s)

READ THESE INSTRUCTIONS FIRST

Write your Name, Class and Index number in the spaces at the top of this page.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

Section A
Answer **all** questions.

Section B
Answer any **one** question on the separate writing paper(s) provided.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

The number of marks is given in brackets [ ] at the end of each question or part question.

At the end of the examination, fasten all the writing paper(s) used securely together.

For Examiners' use only	
Section A	
1	/ 30
2	/ 10
3	/ 10
Section B	
4 or 5	/ 25
Total	/ 75

For Examiner's Use

Section A

Answer **all** the questions in this section.

1 Polyethylene terephthalate (PET) is one of the common polymers used to make plastic bottles. A bacterial species, *Ideonella sakaiensis*, was found to have the ability to break down PET through a two-step process catalysed by two enzymes.

Fig. 1.1 shows the first step in which the enzyme PETase resulted in the degradation of PET into monohydroxylethyl terephthalate (MHET), and Fig. 1.2 shows the second step where the enzyme MHETase breaks down MHET into ethylene glycol (EG) and terephthalic acid (TPA).

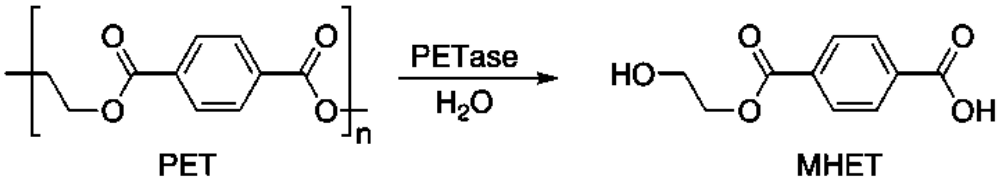

Fig. 1.1

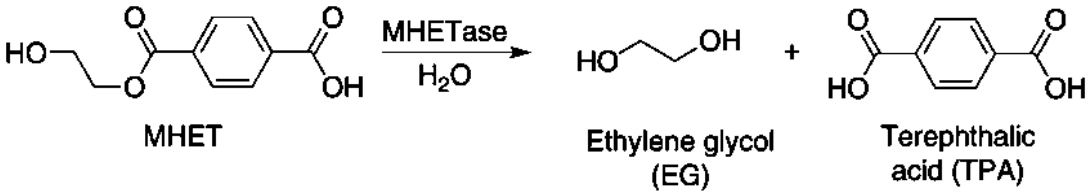

Fig. 1.2

The structures of PETase and MHETase are shown in Fig. 1.3 and Fig. 1.4 respectively.

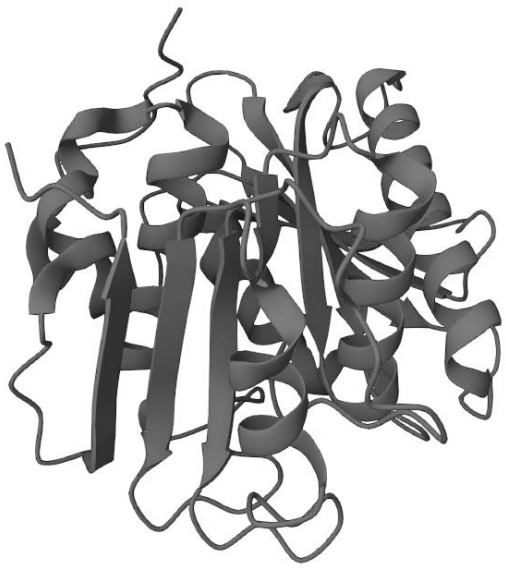

Fig. 1.3

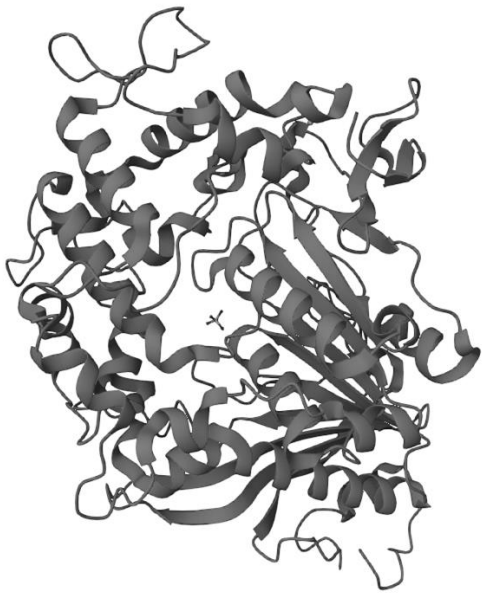

Fig. 1.4

For Examiner's Use

(a) **(i)** With reference to Fig. 1.1 and Fig. 1.2, state the type of reaction that PETase and MHETase catalyse.

…… [1]

(ii) Describe the secondary structures present in PETase and MHETase in Fig. 1.3 and Fig. 1.4.

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[Turn over

During the investigation of the rate of MHETase activity under different conditions, scientists attempted to find the most effective method to quench the enzyme activity (stop further enzymatic action) at the end of an experiment to accurately determine the true enzymatic rate.

However, the solutions used for quenching the enzyme MHETase may also directly react with the substrate MHET and break the bonds in MHET. This unintended chemical reaction gives rise to inaccurate determination of the enzymatic rate.

Table 1.1 shows the results from using four different quenching methods to stop further enzymatic activity of MHETase.

Table 1.1

quenching method	proportion of MHET degraded due to quenching method and not the enzyme / %	success in quenching enzyme activity
Method 1: highly concentrated hydrochloric acid (6 M HCl) at 85 °C	39.40	cannot be determined, as high levels of products from the breakdown of MHET are found
Method 2: 100% methanol at 85 °C	0.25	yes
Method 3: 100 nM PMSF at 85 °C	1.30	no
Method 4: pH 2.5 buffer solution at room temperature	0.00	no

Methanol, used in Method 2, is an amphipathic solvent with the molecular formula, CH_3OH.

Fig. 1.5 shows the structure of phenylmethylsulfonyl fluoride (PMSF), used in Method 3, which acts as an inhibitor that binds at the active site.

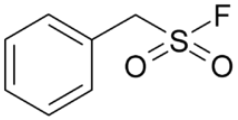

Fig. 1.5

For Examiner's Use

(b) **(i)** With reference to Table 1.1, identify the quenching method that should be used for the experiment and justify your answer.

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(ii) Explain the effect of dissolving an enzyme MHETase in an amphipathic solvent such as methanol.

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(iii) Suggest why PMSF does **not** effectively quench the enzyme activity.

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[Turn over

For Examiner's Use

The scientists examined how proximity of the two enzymes, PETase and MHETase, influences overall rate of MHET degradation.

Chimaeric proteins refer to two or more different proteins that are joined together artificially. Chimaeric proteins of MHETase and PETase were synthesised by using amino acid residues to covalently link the C terminus of MHETase to the N terminus of PETase, giving rise to MP8, MP12 or MP20 (see Table 1.2 and Fig. 1.6). Different linker lengths of amino acid residues resulted in varying mobility between the two enzymes.

Table 1.2

chimaeric protein	number of amino acid (aa) residues found in the linker
MP8	8
MP12	12
MP20	20

Fig. 1.7 shows the rate of MHET degradation with PETase only, MHETase only, MP8, MP12 or MP20 added to the substrate MHET.

Asterisks indicate statistically significant comparisons between MHETase only and each chimaeric protein with *P $\leqslant$ 0.01, **P $\leqslant$ 0.001, and ***P $\leqslant$ 0.0005.

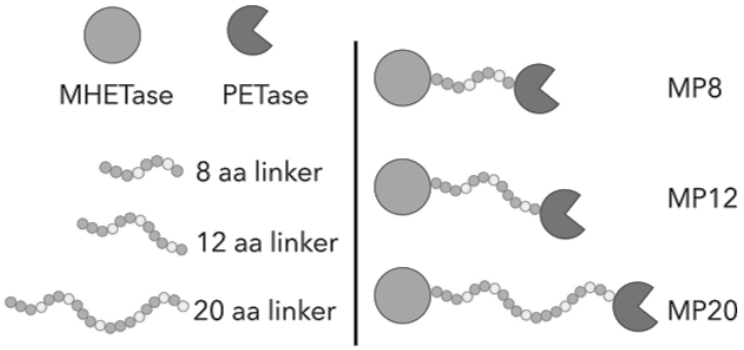

Fig. 1.6

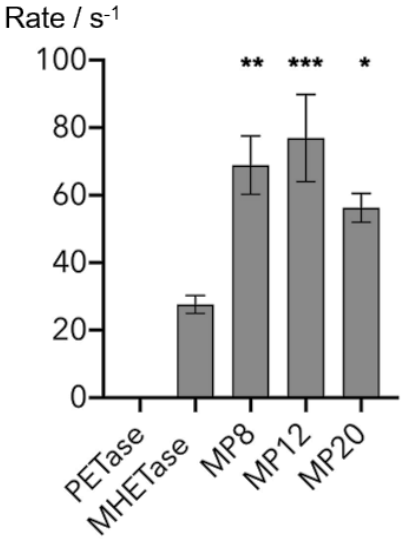

Fig. 1.7

(c) (i) Suggest why an experiment with PETase alone is added to substrate MHET to increase confidence in the results in Fig. 1.7.

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For Examiner's Use

(ii) Fig. 1.7 indicated the statistical significance with $P \leqslant 0.01$ when comparing the rate of MHET degradation with MHETase only and MP20.

Explain what is meant by "significant at $P \leqslant 0.01$".

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[Turn over

MHETase is composed of 611 amino acids.

Table 1.3 shows:

- the sequence of the first 10 amino acids in the primary structure of MHETase;
- DNA triplets in the non-transcribed strand in the gene that codes for the first 10 amino acids in the primary structure of MHETase.

Table 1.3

amino acid position	1	2	3	4	5	6	7	8	9	10
amino acid	leu	leu	gly	asp	phe	phe	arg	lys	ser	lys
DNA triplet	CTG	CTG	GGT	GAT	TTC	TTC	CGG	AAA	TCT	AAA

Table 1.4 shows the triplets of bases in DNA and the amino acids which they code for.

Table 1.4

first base	second base: T		second base: C		second base: A		second base: G		third base
T	TTT	phe	TCT	ser	TAT	tyr	TGT	cys	T
	TTC		TCC		TAC		TGC		C
	TTA	leu	TCA		TAA	stop	TGA	stop	A
	TTG		TCG		TAG		TGG	try	G
C	CTT	leu	CCT	pro	CAT	his	CGT	arg	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	gln	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	ile	ACT	thr	AAT	asp	AGT	ser	T
	ATC		ACC		AAC		AGC		C
	ATA	ile	ACA		AAA	lys	AGA	arg	A
	ATG	met	ACG		AAG		AGG		G
G	GTT	val	GCT	ala	GAT	asp	GGT	gly	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	glu	GGA		A
	GTG		GCG		GAG		GGG		G

For Examiner's Use

(d) Mutations in the base sequence of a gene can affect the primary structure of proteins.

Use the information in Table 1.3 and Table 1.4 to describe the effect on the primary structure of MHETase when there is:

(i) a substitution of the base T with the base A in the middle of the triplet at position 5;

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(ii) an insertion of base G between bases G and T in the triplet at position 3.

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(e) Explain why the genetic code is described as universal.

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[Turn over

For Examiner's Use

(f) With reference to Table 1.4, explain why some mutations have no effect on the primary structure of a protein.

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…… [4]

For Examiner's Use

Fig. 1.8 shows the annual production of plastics around the world, including PET. PET was first invented in 1941 but was only used to make plastic bottles for carbonated drinks since 1973.

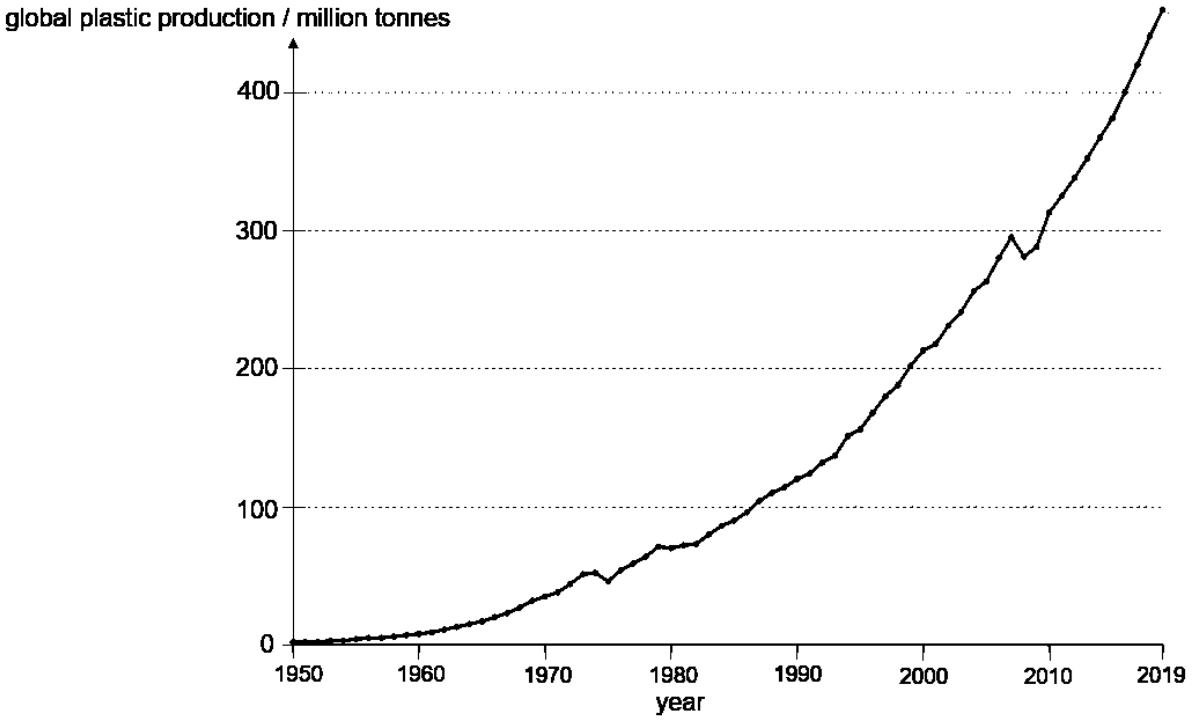

Fig. 1.8

The bacterial species, *I. sakaiensis* was first discovered in 2016, in the soil containing waste discharge from a plastic bottle recycling facility.

(g) Describe the change in global plastic production from 1973 to 2016.

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[Turn over

For Examiner's Use

A colony of *I. sakaiensis* takes about six weeks to completely degrade a sheet of PET. After MHET is broken down into EG and TPA, the products are converted to Krebs cycle intermediates giving rise to carbon dioxide.

As the rate of degradation is too slow to break down the huge amount of plastic waste generated globally, two different methods are used to develop an *I. sakaiensis* strain with more effective PETase and MHETase that will break down PET faster:

- artificial selection, in which the *I. sakaiensis* cells from each generation that degrade PET at the fastest rate are allowed to reproduce; and

- directed evolution, in which different mutations are introduced to create a large number of variants of the PETase gene. These gene variants are then transformed into cells. Cells with the fastest PETase activity are selected and the particular gene variant in these cells will undergo further mutations again. This allows for repeated cycles of creating gene variants and selecting for the cells with the fastest PETase activity.

Evolution through natural selection can take a long time. *I. sakaiensis* was only discovered about 50 years after the production and intensive use of PET.

The two methods, artificial selection and directed evolution, developed *I. sakaiensis* strains that degrade PET faster than wild type *I. sakaiensis* over the past 5 years.

(h) (i) Explain why variation is important in natural selection.

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For Examiner's Use

(ii) Suggest why the two methods, artificial selection and directed evolution, take a much shorter time to develop *I. sakaiensis* strains that degrade PET faster than evolution through natural selection.

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(iii) Using a named species concept, explain why the cells that are grown from these two methods are considered *I. sakaiensis strains* and not a different species from wild type *I. sakaiensis*.

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[Total: 30]

[Turn over

For Examiner's Use

2 Patients infected with influenza virus often present common symptoms such as runny nose, blocked nose and sore throat.

(a) Explain how the influenza virus causes such symptoms.

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In the United States (US), the flu season usually occurs in the fall and winter, which is between September to March. People are encouraged to go for their seasonal flu vaccination yearly.

(b) **(i)** State the first class of antibody secreted by the plasma cells after vaccination.

…….. [1]

(ii) Complete Table 2.1 to show the type of bonds found in the antibody mentioned in **(b)(i)**. Use a "yes" to indicate presence and "no" to indicate absence. The first two blanks have been filled in for you.

Table 2.1

type of bond	presence in first class of antibody secreted after vaccination
hydrophobic interactions	**yes**
hydrogen bonds	**yes**
ionic bonds	
disulfide bridges	
peptide bonds	
phosphodiester bonds	

[1]

For Examiner's Use

The vaccine for seasonal flu is designed to be effective against a few strains of influenza virus that are predicted for the next season. There was also another vaccine that targeted the prevailing flu virus (H1N1) which emerged in April 2009.

Fig. 2.1 shows the number of vaccinations administered for seasonal flu and H1N1 in the US between August 2009 and March 2010. Vaccination data was collected weekly.

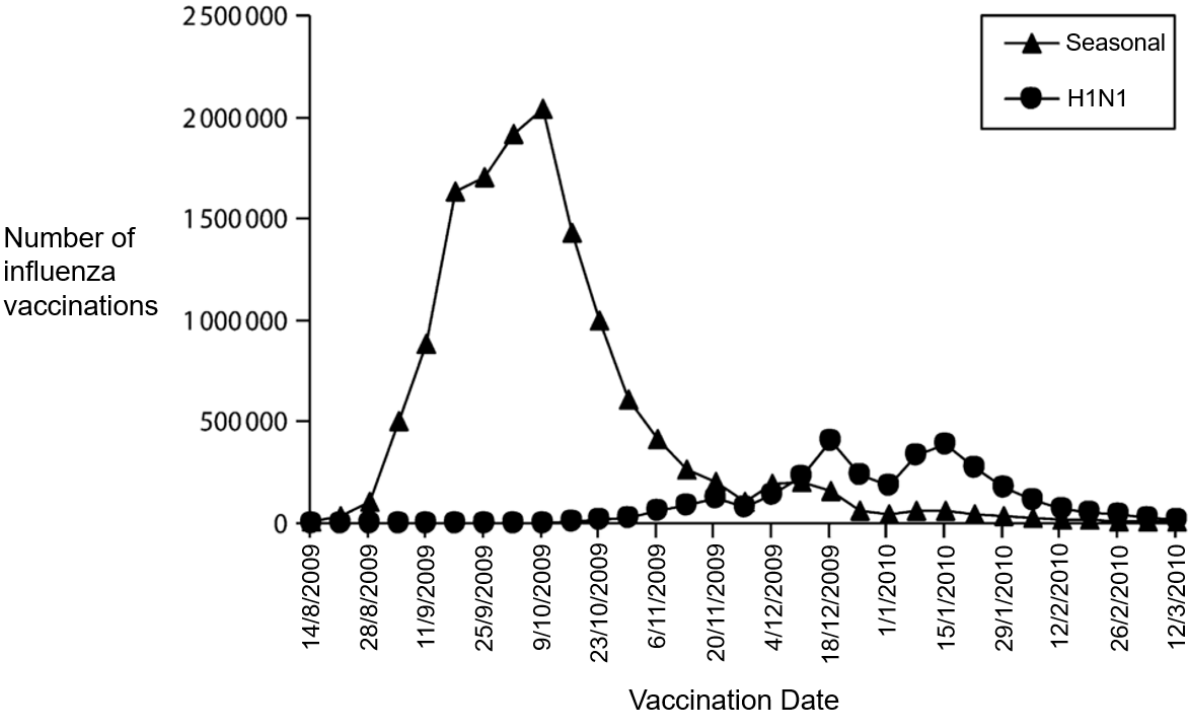

Fig. 2.1

(c) **(i)** Describe the change in the number of vaccinations administered for seasonal flu over the period.

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(ii) Suggest why the number of vaccinations administered for H1N1 remained at zero for the first 8 weeks.

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[Turn over

For Examiner's Use

Number of vaccinations administered for seasonal flu were not as high as expected due to claims that flu vaccinations caused Guillain-Barré Syndrome (GBS).

GBS is a rare autoimmune disorder where the body's immune system damages nerve cells.

A study was conducted to determine if flu vaccinations cause GBS. The number of actual cases of GBS that occurred after vaccination were tracked to give the observed rate and compared against a predicted rate. The predicted rate is computed based on the predicted number of GBS cases if GBS was caused by flu vaccinations.

Fig. 2.2 shows the results of the study for seasonal influenza vaccination.

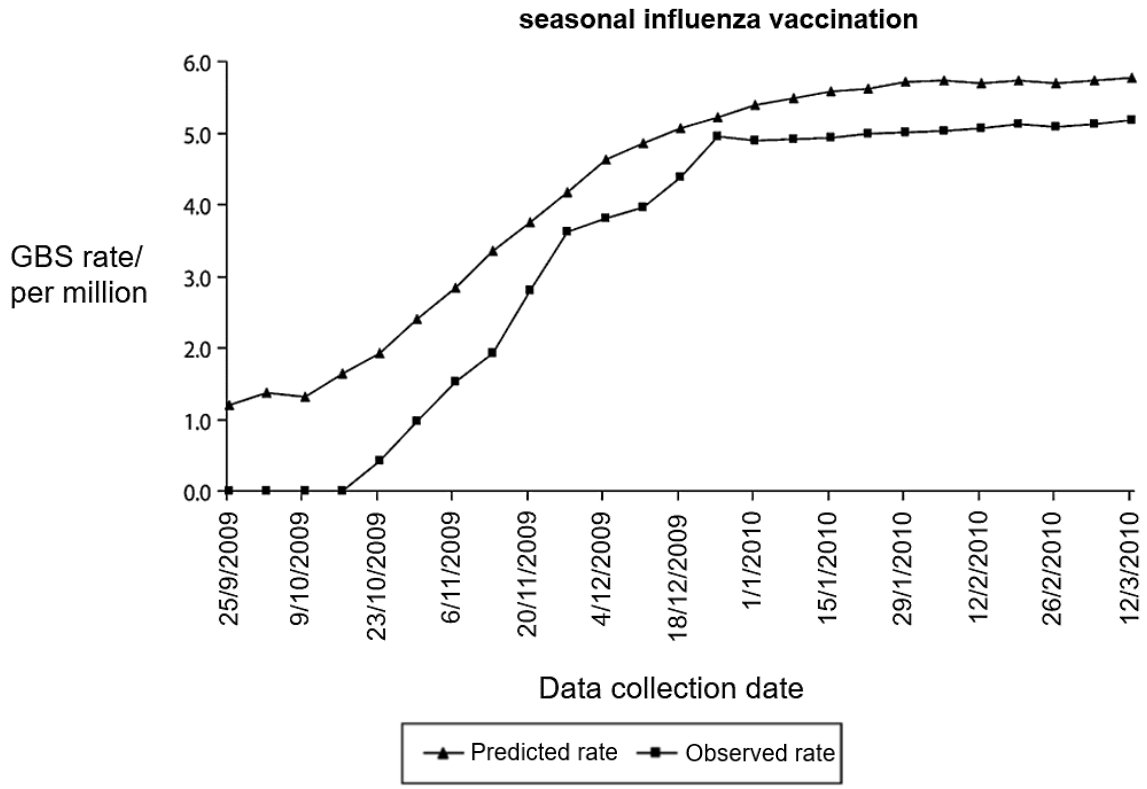

Fig. 2.2

(d) With reference to Fig. 2.2, explain why the claim that flu vaccination causes GBS is untrue.

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[Total: 10]

For Examiner's Use

3 Fig. 3.1 shows the regions where wheat, rice and maize are cultivated in the world.

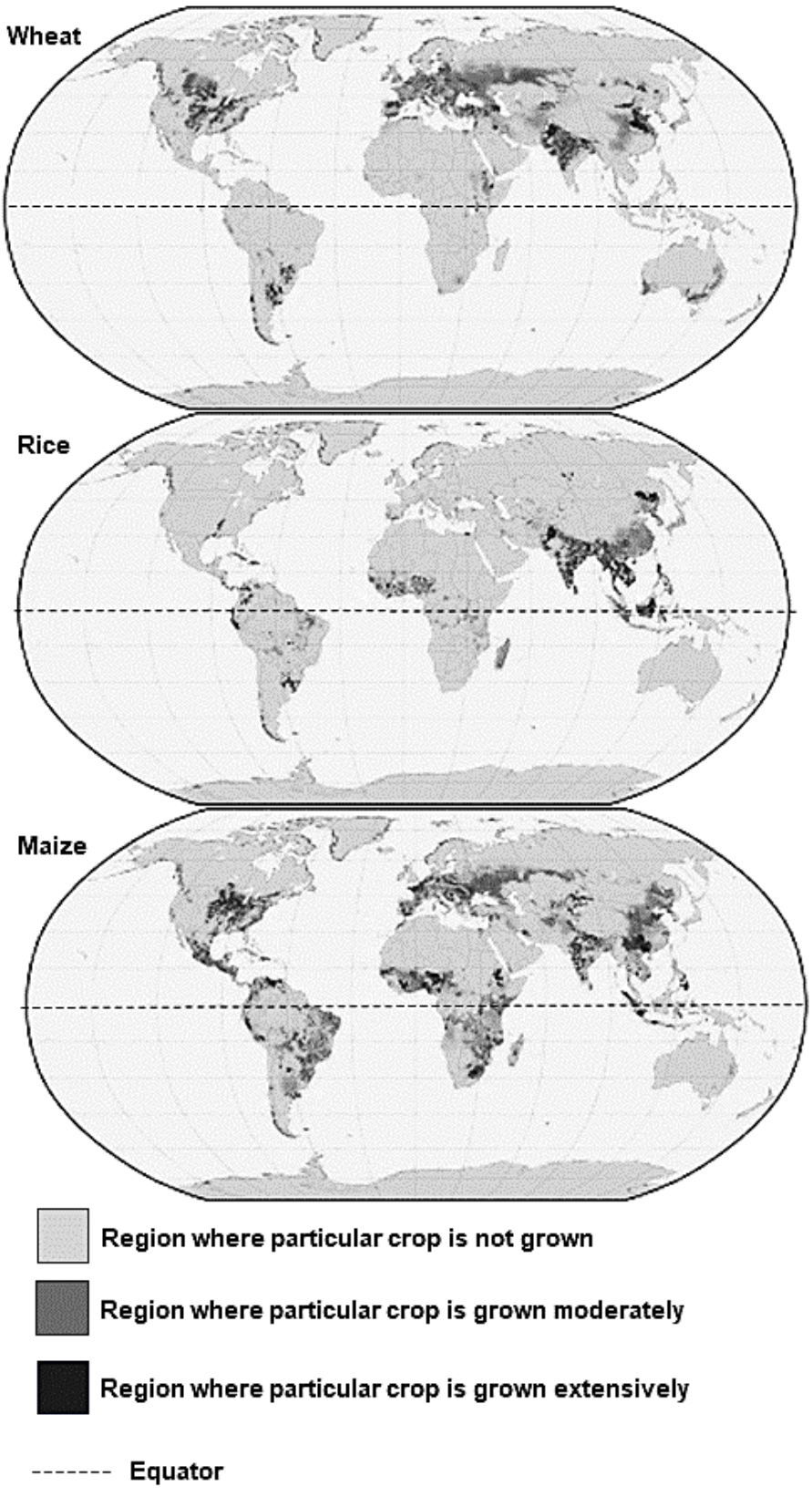

Fig. 3.1

[Turn over

Fig. 3.2 shows the projected total global crop yield losses of wheat, rice and maize in megatonnes per year (Mt/yr) due to increased insect pests' metabolic activities (triangles on each graph) when temperature rises from 0 to 5 °C (reflected on x-axis) above pre-industrial levels.

The dashed horizontal line on each graph reflects the current amount of crop losses as a result of insect pests.

An estimation of crop population growth rate is also reflected (as ovals on each graph) to show the predicted trends across the range of temperature increase.

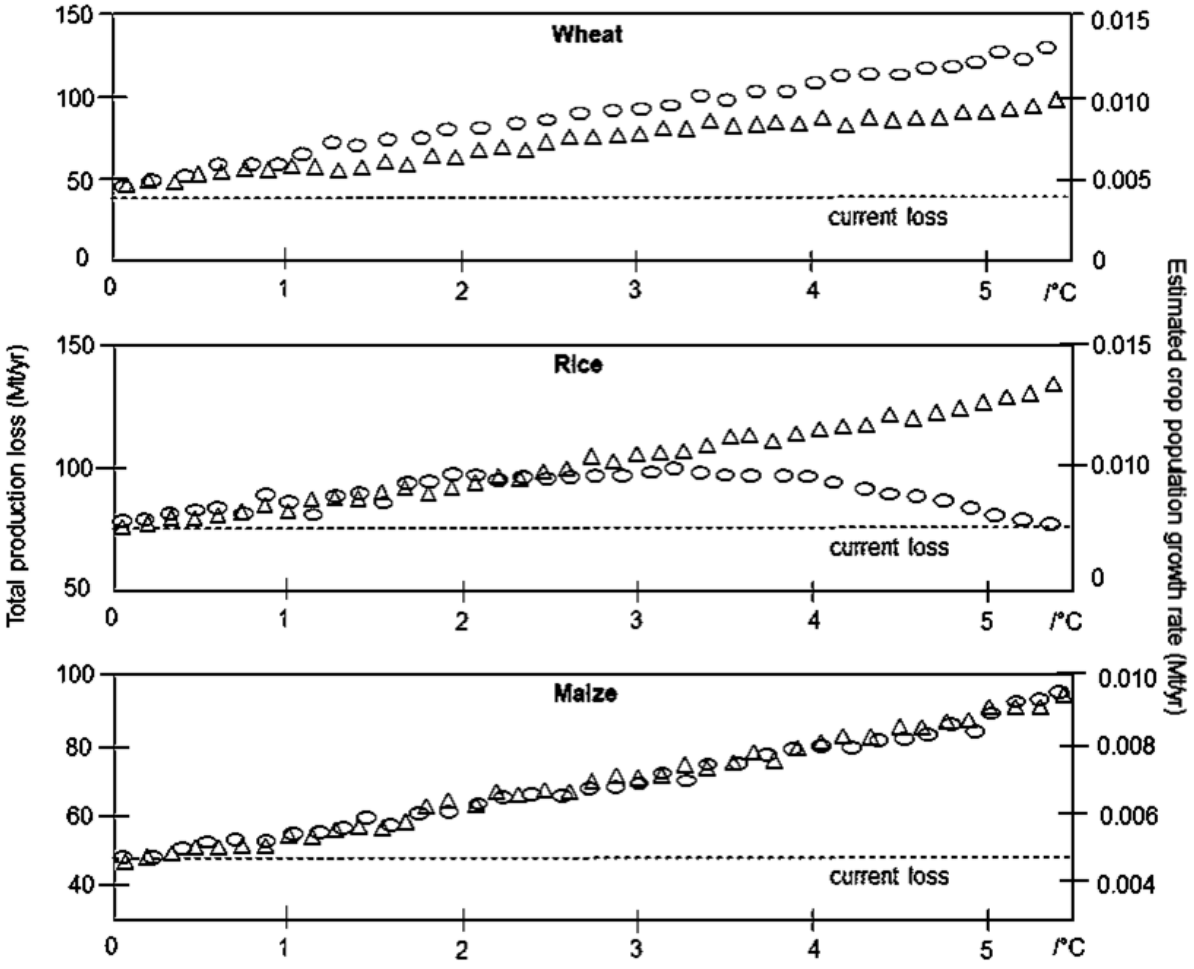

Fig. 3.2

For Examiner's Use

(a) **(i)** Calculate the percentage increase in pest-related crop yield losses for rice and maize at a temperature rise of 5 °C above pre-industrial levels. Show your working.

[2]

(ii) With reference to Fig. 3.1 and Fig. 3.2, suggest reasons for the difference in the percentage increase in pest-related crop yield losses between rice and maize.

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(iii) Given that the trend shown in Fig. 3.2 continues, calculate the total crop yield loss of wheat due to pests if temperature increases to 6.5°C above pre-industrial levels. Show your working.

[2]

[Turn over

For Examiner's Use

(b) Increase in insect pests due to climate change can contribute to a change in biodiversity on a cropland and subsequently impact crop yield.

(i) List two examples of organisms, excluding insect pests, that can increase the biodiversity in a particular cropland and explain how these organisms can change crop production as temperature becomes more permissive for other species to migrate to the area in Table 3.1. The first example of organisms has been filled in for you.

Table 3.1

	organisms (excluding insect pests) that increase the biodiversity in a particular cropland	impact on crop production
Example 1	**butterflies and birds**	…… …… …… …… ……
Example 2	…… …… …… …… ……	…… …… …… …… ……

[3]

(ii) Suggest why dietary habits can be affected by the decline of crop production due to climate change.

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…….. [1]

[Total: 10]

For Examiner's Use

Section B

Answer **one** question in this section.

Write your answers on the separate writing paper provided.

Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.

Your answers must be in continuous prose, where appropriate.

Your answers must be set out in parts **(a)** and **(b)**, as indicated in the question.

4 **(a)** Explain how viruses challenge the cell theory while prokaryotes and eukaryotes comply with the cell theory. [14]

(b) "Proteins are the most important biomolecules for cell signalling."

Discuss the validity of this statement. [11]

[Total: 25]

5 **(a)** Explain how errors during various stages of mitotic and meiotic cell cycles can lead to cancer. [14]

(b) Stem cell therapy involves the differentiation of stem cells into specialised cells before transplanting into patients. However, complete differentiation may not have occurred in every cell, and there could still be undifferentiated cells remaining within the transplanted cells.

Discuss the possible outcomes in a patient who received transplanted cells with the presence of undifferentiated stem cells. [11]

[Total: 25]