Measuring Metabolism Using O_2 and CO_2[1]

Biology 390 – Physiology

Class note readings: Review the section on aerobic metabolism. Be sure that you understand the use and calculation of RQ.

Introduction and Review From Lecture: Metabolism is the sum of all chemical reactions occurring in an organism. It is an extremely important physiological characteristic since it relates to the rate of nearly every major process in the body. We assume that one of the most important factors in the evolution of metabolic rate is maximization of lifetime reproductive success given a certain availability of energy and other resources.

The measure of metabolism that is most related to reproductive and growth rates is the resting, standard, or basal rate of metabolism (these all have slightly different meanings which will be covered in class). This lab will focus on these measures of metabolism as they are influenced by body size. Next week's lab will consider the influence of temperature on resting metabolism and later labs will investigate the relationships between movement and metabolic rate.

Metabolism is generally reported as either work (how much energy did an animal use) or more commonly as power (how fast did an animal use energy or what was the rate of its chemical reactions). Most of the measurements we make this semester will be power measurements; although late in the semester we try to quantify the cost (in energy) of doing certain tasks. As was discussed in the third class packet, we usually report metabolism as either:

- a rate of energy release ($\dot{Q}$, given as watts or as cal/time)

or more commonly as a **rate of respiratory gas use,** either oxygen consumption

- $\dot{V}O_2$, usually given as either a volume of oxygen used per unit time or $\dot{M}O_2$ which is the number of mols of oxygen used per unit time

or

- $\dot{V}CO_2$, the volume of CO_2 production per unit time or $\dot{M}CO_2$ the number of mols of CO_2 produced per unit time.

In this lab we will learn the principles of measurement of respiratory gases and we will also learn how to calculate metabolic power.

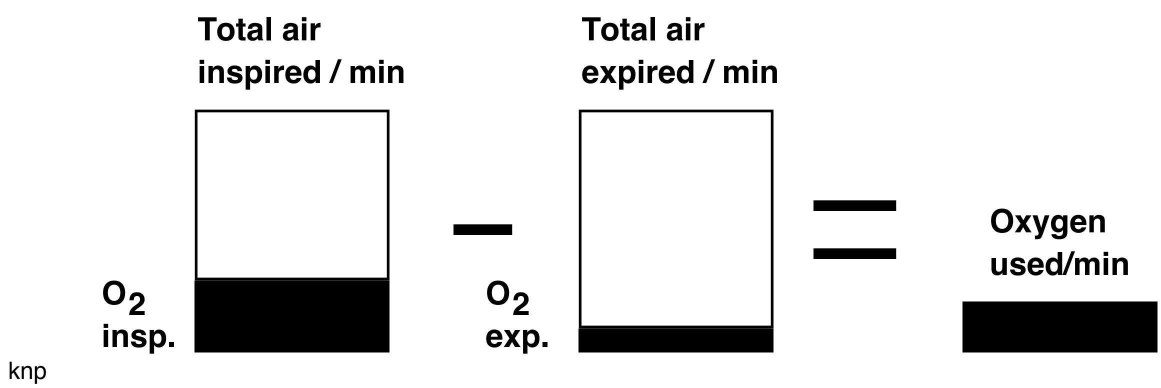
Measurement of Metabolism Using Respirometry: In lecture packet #3 we learned that if respiratory gases are measured, we can estimate the extent of aerobic metabolism. This section goes over the basic procedures involved in measuring respiratory gases. Two general techniques are used. One is the **measurement of gas fractions of expired and inspired air** and the other is **manometry**.

I. Measurement of Metabolic Rate Using Gas Fractions:

In these types of measurements there are three pieces of equipment that are required:

- a gas analyzer. This is a device that will determine the fraction or partial pressure of a respiratory gas in a sample. Examples are **solid-state oxygen analyzers** such as the **Sable Systems FC-**1 and the **Ametek S3-AII** that we will use.[2] For CO_2 the usual method is **infrared spectrometry**[3].
- Some sort of device to collect air (especially expired air) for gas fraction analysis. In our experiments we will use either a **collection bag** (called a **Douglas Bag**) or we will seal the animal in a closed container (**metabolic chamber**) and then remove samples of the air from that container.
- Some sort of way to measure the rate at which the gas sample is produced. We will use either a **spirometer (vitalometer)**[4] or the container the animal is sealed in. In both cases if we record the time over which the sample is obtained, we can calculate a rate.

General Principle of Respiratory Gas Measurements: When using gas fraction respirometry, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are determined using the same general principle: the change in respiratory gases between inspired and expired breaths per unit time is the rate of gas consumption. To get this measurement we need to follow the procedure shown in the cartoons on the top of the next page:



The inspired and expired air volumes are represented by the large boxes; the dark portions of each box are the proportion of the volume that is oxygen (or by analogy, CO_2). Exactly the same procedure would be used for CO_2 except that it would be expired CO_2 minus inspired CO_2 since CO_2 is produced in the body.

Using oxygen as an example, the parameters that we need to measure include:

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[2] In this device, oxygen induces a change in redox state of a heated zirconium oxide crystal; this change in redox leads to a change in electrical properties that is proportional to the amount of oxygen present.

[3] CO_2 absorbs strongly at certain infrared (IR) frequencies that are not absorbed by other substances that are likely to be in the air. Thus, if air is passed through a vessel illuminated by IR, the amount of IR light passes through can be determined using a phototube and the concentration of CO_2 by Beers and Lambert's laws -- the result is that CO_2 can be determined by a method that is similar to that used in any spectrophotometer.

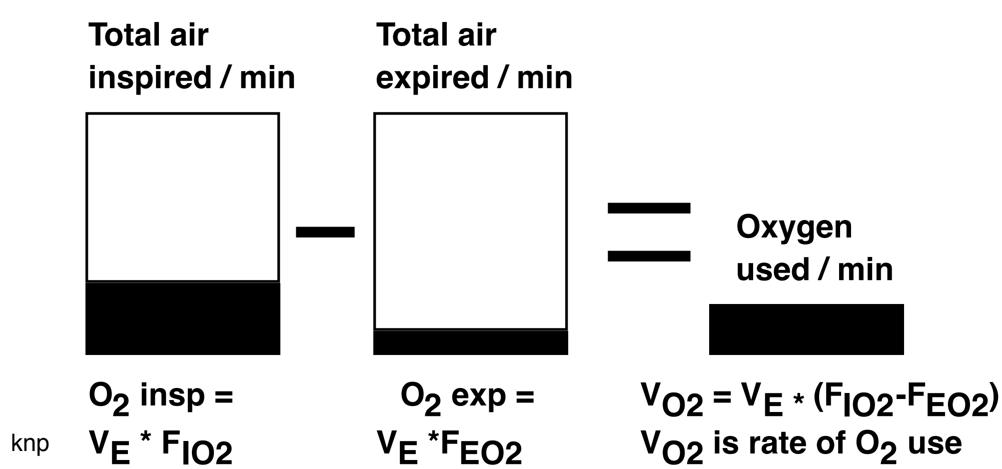
[4] These are calibrated collection vessels. As expired air is collected in a spirometer, the volume that has been collected is continuously recorded.

(i) the volumes of air inspired and expired per unit time. <u>We assume that these are equal</u> and we will refer to them as the **expiratory minute volumes,** $\dot{V}_E$. In our experiments with people, we will measure this by using a spirometer and clock.

> **!** We use expiratory volumes since they are somewhat easier for us to measure than inspired volumes (you will eventually learn how to measure both).
>
> **?** In fact, what is the only situation where inspiratory minute volume, $\dot{V}_I$, equals $\dot{V}_E$?
> Hint: it has to do with RQ. Think about the definition of RQ.

- **(ii) The fraction of inspired air that is oxygen ($F_{I\,O2}$).** Fractions are simply percentages divided by 100. Since the percentage of oxygen in room air is very constant at about **20.94%**, we will assume that $F_{I\,O2}$ **= 0.2094.**
- **(iii) The fraction of expired air that is oxygen ($F_{E\,O2}$).** We will split the expired air from our subjects. As was mentioned above, some will be routed to the spirometer and used to determine volume of air expired per minute. However, most will go to a large collection bag, called a **Douglas Bag**. We will analyze the expired gas in the Douglas Bag using the O_2 analyzer to find F_{ECO2} .

Now, since the rate at which oxygen is inspired is equal to the total rate that air is inspired ($\dot{V}$) times the fraction of that air that is oxygen ($F_{I\,O2}$) we can calculate the amount of oxygen inspired per minute ($\dot{V}_{I\,O_2}$) using these two measures. Using similar measures, we can discover the amount of oxygen expired per minute ($\dot{V}_{E\,O_2}$). The rate of use is therefore the difference between the two. Below is an illustration of this using the same figure as before and the appropriate equations:



The equation we will use is a little different than the one given in the equation in the figure above. While I do not want to burden you with the derivation (it takes several pages), suffice it to say that it takes into account differences in RQ (and therefore differences in inspired and expired minute volumes). **Know this equation and the general derivation presented above**:

1. $$\dot{V}_{O_2} = \dot{V}_E \ * \frac{(F_{IO_2} - F_{EO_2})}{(1 - F_{EO_2})}$$

where $\dot{V}_{O_2}$ is the rate of oxygen consumption, $\dot{V}_E$ is the volume of air the subject breathes in one min (minute volume), $F_{I\,O2}$ is the fraction (percentage divided by 100) of inspired air that is oxygen (i.e. 0.2094) and F_{EO2} is the fraction of expired air that is oxygen (i.e., the percentage measured with the O_2 analyzer).

Rate of Carbon Dioxide Production ($\dot{V}_{CO_2}$): We will measure $\dot{V}_{CO_2}$ by an analogous technique: find the fraction of carbon dioxide in inspired and expired air and also determine the expired minute volume, $\dot{V}_E$. (In fact we will use the same sample of air for both the carbon dioxide and oxygen analysis (see below)). The equation to determine carbon dioxide production is rather complex and we need not worry about its derivation:

2. $$\dot{V}_{CO_2} = \frac{(\dot{V}_E \ * \ (F_{E\,CO_2} \ - \ F_{I\,CO_2}) \ - \ F_{I\,CO_2} \ * \dot{V}_{O_2}}{(1 - F_{I\,CO_2})}$$

However, it can be simplified since we will assume F_{ICO2} to be 0. Believe it or not, even with all of the concern about global warming, there is really very little CO_2 in fresh air -- about 0.03% (thus, $F_{I\,CO2}$ = 0.0003, this shows what a potent greenhouse gas it is -- moreover, think what a hard time plants have getting the CO_2 they need!). So, if $F_{I\,CO2}$ is zero, it reduces equation #2 to the form we will use:

3. $$\dot{V}_{CO_2} = \dot{V}_E \ * F_{ECO2}$$

II. Obtaining $\dot{V}_E$ Measurements with the Vitalometer

The vitalometer we will use (a.k.a. spirometers) have maximum volumes of 10L. Most of our spirometers will make a paper record on a rotating drum known as a **kymograph**. They can also be hooked up to a computer but we will probably not do that just yet. When the kymograph is used, the record is obtained by turning a switch below the drum -- this causes the drum to rotate at a certain speed (two speeds are available-- at **high speed** it takes **1 sec to go between the time marks** while at l**ow speed it takes 4 seconds**).

You should always try to take advantage of nearly the entire volume of the spirometer. In other words, even though $\dot{V}_E$ might simply be measured at rest by noting the expired volume after one minute of breathing, it would be better to record longer and then convert whatever volume was recorded to a minute volume. And when subjects exercise and have large $\dot{V}_E$ you will almost certainly see them fill the volume in less than 1 minute and will need to make some conversions to get a true $\dot{V}_E$.

Here is how to determine $\dot{V}_E$:

- Route the air to the Douglas bag (or simply the room -- disconnect the Douglas bag until you start the experiment -- see below)
- Let all of the air out of the spirometer by removing the hose where it attaches to the spirometer.
- Reconnect the hose
- When ready, and without the subject knowing, turn the three way valve to route air to the spirometer.
- **Turn the kymograph speed to LOW** (there will be other times when you wish to use high speed). Recall that this means there are 4 sec between each time mark (you should check to be sure this is accurate!)
- **When you think that the next expired breath is likely push the volume in the vitalometer close to 10 L, get ready to turn the three-way valve to re-route air to the Douglas Bag.**
- **The instant that the next expired breath starts** (you will see the spirometer bell start to move upwards) **re-route the air to the Douglas Bag**. It won't matter if some air goes into the vitalometer.
- Determine total time between the start of the first and start of the last breath. **Whatever you do, be certain to time using complete breathing cycles. Determining a cycle is easy -- each time the subject breaths out the record moves; when the subject inhales or rests, there is no movement.** So, be sure to time from start to start. **Note**: at rest failure to do this over 10 liters is not likely to result in a large error -- however, when subjects exercise (high $\dot{V}_E$) significant errors are certain.
- **Record the total time in seconds** and the **number of breaths and the average** $\dot{V}_E$.
- You should **estimate** $\dot{V}_E$ **at least 5 different times** over the time period when you are filling the Douglas Bag.

! Very Important Note: I cannot emphasize too much the importance that full breathing cycles (start of expiration to start of expiration) are used to measure $\dot{V}_E$.

? How would failure to do this result in inaccurate estimates of $\dot{V}_E$?

The **calculation of** $\dot{V}_E$ **is straightforward**. What you have just done is find the volume or air expired in some period of time. Since $\dot{V}_E$ always has units of volume (usually liters) per time (usually minutes), then:

4. $\dot{V}_E$ = Volume expired (liters) * $\left\{\frac{60}{\text{time to collect air in seconds}}\right\}$

> **!** Breathing rate (***f***) can be calculated by a similar formula except instead of volume use the number of breaths.

The $\dot{V}_E$ that you find this way will be under ambient conditions. More formally, we say that this gas is collected under ***ATPS*** (for **ambient temperature and pressure, saturated**). Note that by the time the air volume is measured it has cooled from body temperature to approximately room temperature, note that it is saturated with water vapor both since it came from the body and also since it was cooled over water in the vitalometer, and note that it is at ambient pressure. We will need to correct this volume to standard conditions, ***STPD*** (for **standard temperature and pressure, dry air**). This can be done using the following formula. You should either know this formula or better yet, be able to deduce it from what you know about the gas laws from chemistry. We will go over it in lab:

5. $$\dot{V}_{E(STPD)} = \dot{V}_{E(ATPS)} * \frac{273}{(273+T_a)} * \frac{(P_b - P_{H_2O})}{760}$$

where $\mathbf{T_a}$ is the temperature of the gas in the spirometer, P_b is the barometric pressure (we will **assume that it is 760 torr**), and P_{H2O} is the partial pressure of water at the temperature of the air in the vitalometer (look-up this value on the table attached to these notes).

Take all of your values of $\dot{V}_E$ at ATPS **average them**, and then **convert the average to STPD.**

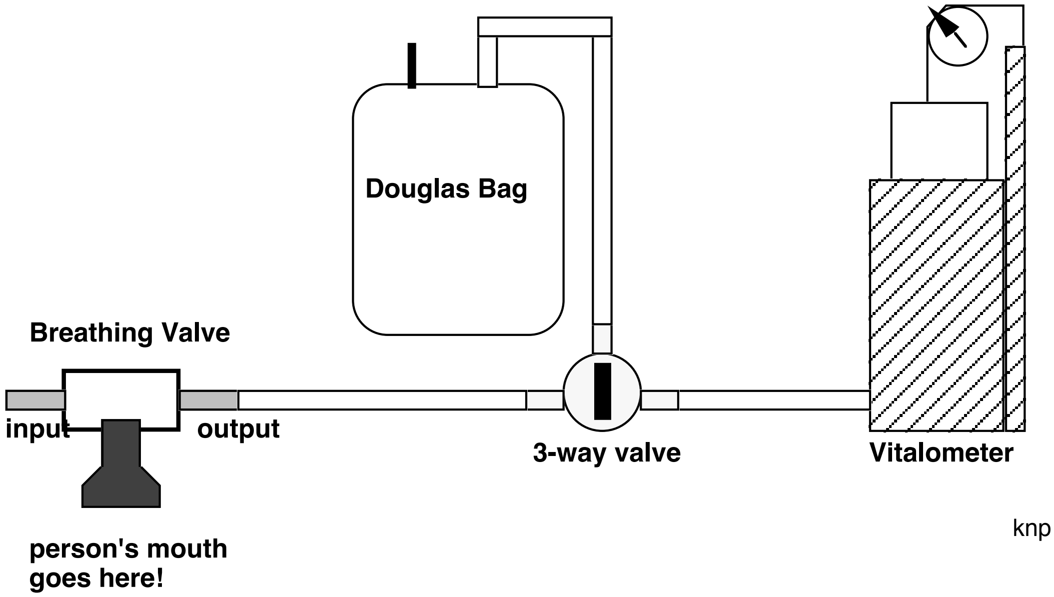
Use the STPD $\dot{V}_E$ for your calculation of $\dot{V}O_2$ (see below).

III. Measurement of Gas Fractions

The gas contained in the Douglas bag is used to find F_{EO2} and F_{ECO2} . You will use the oxygen and carbon dioxide analyzers to get these numbers. Your instructor will demonstrate how to use the machines. The most important thing to remember is that they will give you values as **%O_2** and **%CO_2**. **You must convert these values to fractions**; simply divide the percentages by 100.

IV. Human Metabolic Rates: Experimental Protocol

We will collect approximately 100 L of expired gas from our subjects. Our gas collection set-up will look like the diagram below. The subject breathes in through a **2 way respiratory valve**. One side of this valve is opened to the room and allows the subject to inhale; when the subject exhales her/his breath is prevented from leaving the inhale side and instead exits via the other side of the valve. The gas is then routed via a **three-way airstream control valve** to either the large collection bag (called a Douglas Bag -- the ones we use have a volume of about 100L) or to a vitalometer so that we can measure the expiratory minute volume $\dot{V}_E$. Gas routing is controlled by a three-way valve.



The subject will rest comfortably and **face away from the apparatus**.

- Initially have the person breathe through the system but with the hose **NOT** connected to either the Douglas bag or the spirometer.
- After a few minutes of this, make full connections and begin alternately collecting air in the bag and measuring $\dot{V}_E$ using the vitalometer (see below).

? Why is it especially important to have the subject face away from the apparatus?
? Why are the initial few minutes of breaths not collected? (ans. below)

It is vital that the subject become accustomed to and relaxed with the apparatus. This is to prevent hyperventilation (see following box). You will be told how to identify hyperventilation in lab but a good rule of thumb is that **if someone is at rest and their $\dot{V}_E$ is greater than 4 to 6 L/min (depending mostly on their size), they are almost certainly hyperventilating** and you should start over.

About Hyperventilation and RQ: We will have the opportunity to study hyperventilation in some detail later this semester in both lab and lecture. However, you need to know a bit about it now. Someone hyperventilates when they bring more air into their alveoli than they need to maintain normal steady-state values of O_2 and CO_2. This increases the diffusion gradient for O_2 into the blood and for CO_2 out of the blood. For reasons that will be covered later in the course, we will see that hyperventilation has very little effect on $\dot{V}_{O_2}$. However, it does have a large effect on $\dot{V}_{CO_2}$ because of the large stores of CO_2 (mostly as bicarbonate) that are found in the blood and tissues. As we will learn shortly in lecture, if one increases the difference in partial pressure between blood and alveolar air, much CO_2 will diffuse out and $\dot{V}_{CO_2}$ will appear to increase. In fact ,the rate of production of CO_2 in the tissues is still about the same but the rate of exchange is higher and so ***apparent*** $\dot{V}_{CO_2}$ increases.

For this reason, if someone is hyperventilating (or recovering from hyperventilation) any "RQ" measured at the exchange site (*i.e.*, the start of the respiratory tract) will not be accurate. Thus we must avoid hyperventilation.

V. Spreadsheet Calculation of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$

Using the gas fractions you obtained from the Douglas bag and the $\dot{V}_E$ @STPD (see equation #5), use equations #1 and #3 to calculate the **rates of oxygen consumption and carbon dioxide production**. **Do this using a spreadsheet** (see handout on spread sheets for this lab). Report this number along with your **subject's sex, height in meters and weight in kg**. Also calculate your subject's **RQ**.

No calculators allowed in this lab! -- I want to encourage use of Excel.

Appendix I -- Calculations Spread Sheet for Gas Respirometry

During class, go to a computer and open Excel. You are going to set up a worksheet that you will use the rest of the semester when you need to calculate metabolic rates. A suggested pattern is shown below:

MR Calc SS

	A	B	C	D	E	F	G	H
1	Metabolic Rate Calculations Sheet							
2								
3	subject							
4	mass (kg)							
5	ht (m, optional)							
6	Ta							
7	Pb							
8	PH2O							
9	cf, ATPS->STPD							
10	FEO2							
11	FECO2							
12								
13		Expired Vols	Time	Ve(ATPS)	Ve(STPD)	VO2	VCO2	RQ
14								
15								
16								
17								
18								
19								
20								
21				average				

- You will enter the following data into the spreadsheet: ht, mass, atmospheric pressure (torr), P_{H2O} (torr), T_A (in ^oC), $\dot{V}_E$ **(multiple readings for each subject -- see protocol above)**, F_{EO2}, and F_{ECO2} (single readings for each subject).
- Find the average value of $\dot{V}_E$.
- **Enter the formula needed to take the pressure and temperature data to find the cf needed <u>to convert an ATPS volume to an STPD volume</u>**. Refer to **eq. #5** if you need help.
- **Convert the $\dot{V}_E$ at ATPS to STPD** .
- Use this value and appropriate gas fractions to **calculate the $\dot{V}O_2$ and $\dot{V}CO_2$ in the next two cols.**
- Finally, use these values to **calculate RQ.**

<u>Make sure I see you sheet before you use it for data analysis.</u>

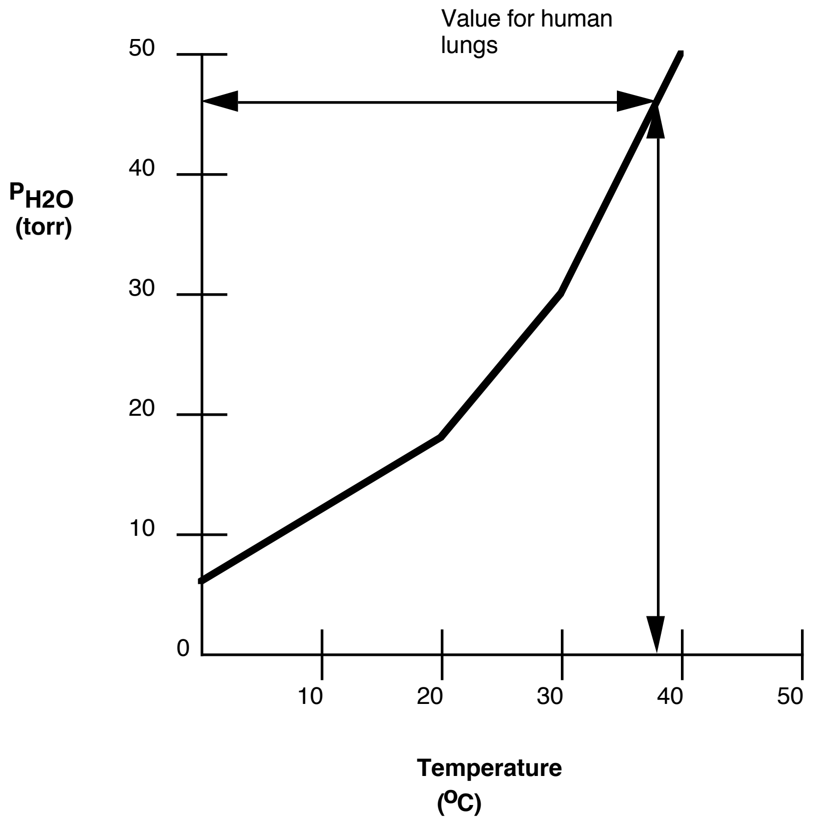
<u>Please save the spreadsheet onto your computer and a floppy.</u>

Appendix 2 -- Vapor Pressures

Vapor Pressure and Temperature under Saturated Conditions

Temp (°C)	P_{H2O} (torr)
20	17.5
21	18.6
22	19.8
23	21.1
24	22.4
25	23.8
26	25.2
27	26.7
28	28.4
29	30.0
30	31.8
31	33.7
32	35.7
33	37.7
34	39.9
35	42.2
36	44.6
37	47.1
38	49.7
39	52.4
40	55.3

knp



Assume that the relative humidity in the classroom (or cold room later in the semester) is 50% -- that is, that the air is half-saturated. Thus, if the temperature is 29°C the table says that saturated air has a P_{H2O} of 30 torr and since we assume a relative humidity of 50%; you would use a value of 15 torr P_{H2O} in your ATPS -- STPD calculations.